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THE RELATIONSHIP OF KIDNEY FUNCTION TO THE GLUCOSE UTILIZATION OF THE EXTRA ABDOMINAL TISSUES

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The study of the carbohydrate metabolism of the hepatectomized and of the eviscerated animal has a very important bearing on our knowledge of metabolism in general, and particularly the metabolism in diabetes. The review by Peterson (1) cites the work that has been carried out in this field and gives the deductions derived from it as regards carbohydrate metabolism. The usual assumption made in applying the results obtained with these preparations to the preoperative carbohydrate metabolism is that the glucose utilization of the tissues remaining after the operation continues the same as it was in them in the intact animal. This assumption has been attacked by those who claim that such an operation must alter the metabolism of the animal. Nothing has been produced to back up this contention and it remains a hypothetical objection. We can say that this method of studying tissue metabolism is the best available, and gives definite presumptive information which becomes conclusive when it agrees with the results obtained by other methods such as the study of metabolism of tissue slices, and of arterio-venous differences.

The properly hepatectomized animal is, for several hours after operation, in a condition in which physiological functions (other than those connected with the liver) remain normal when measured quantitatively. The oxygen consumption remains at the preoperative level (2, 3, 4); there is a drop of about 10 per cent or less which is undoubtedly that of the liver that has been removed. The R.Q. changes little (3, 4, 5) and this small change is attributable to the liver. Respiration, circulation and temperature remain normal, kidney function keeps up, absorption from the gastro-intestinal tract continues and reflexes remain normal (6, 7). In as far as

we can measure function the physiological processes of the surviving systems are normal. It is then, somewhat of a presumption to suppose that the nature of the metabolism of the surviving tissues (such as muscle, brain, supporting tissues and the other organs of the body) is suddenly altered by the removal of the liver. However, the animal must be otherwise normal and the physiological functions enumerated above must carry on as usual. If the animal does not maintain these functions in a proper manner, or if any of them are interfered with artificially, it becomes unsafe to assume the continuance of the normal carbohydrate metabolism after hepatectomy. In this paper we will show how interference with one of the body functions (kidney activity) has a profound effect on the sugar utilization of the hepatectomized and of the eviscerated animal. In the course of our investigations of the carbohydrate utilization of hepatectomized animals, we had removed in some the kidneys along with the liver. Such animals we have found to have sugar utilization rates double or more the utilization rates of animals with only the liver removed. This led us to investigate specifically this relationship.

Methods. Rabbits were used throughout. Hepatectomy was done by a modification (7) of the Markowitz-Soskin method (8). Evisceration was carried out by the method of Drury (9). Glucose requirement was determined by the method (10) already described which is essentially the amount of glucose that has to be given intravenously per unit body weight to maintain the blood sugar at a normal level. Frequent blood sugar determinations are carried out and adjustments in the injection rate are made as indicated by the blood sugar changes.

Results. We removed from a three day fasted rabbit both the liver and the kidneys and followed the glucose utilization rate thereafter by the method used in this laboratory (10). The glucose requirement rose from a value of 78 mgm/kgm.hr. 2 hours after operation to 270 mgm/kgm.hr. 5 hours later. In another rabbit we ligated both ureters after evisceration and followed the glucose requirement; it rose from 67 mgm/kgm.hr. 2 hours after operation to 145 mgm/kgm.hr. 5 hours later. This abrupt rise of sugar need gives a marked contrast between these preparations and those hepatectomized (11), or eviscerated (9) and with normal kidney function, which have quite steady rates of utilization for at least the first 10 hours after operation.

In the next type of experiment we first determined the glucose utilization and after 3 hours stopped renal function and followed the rate thereafter. In these animals at the time of operation we laid loose ligatures around the kidney pedicles and brought the ends out through the skin of the back. Kidney function was stopped when needed, by tying these threads down firmly.

The following protocol illustrates the manner in which the experiments

were carried out. The figure after "Rate of injection" refers to the rate during the preceding hour.

9:30 Operation finished.

9:58 Blood sugar 90.
10:45 Blood sugar 98. Rate of injection 128 mgm. per kilo per hour
11:46 Blood sugar 109. Rate of injection 118 mgm. per kilo per hour
12:46 Blood sugar 115. Rate of injection 106 mgm. per kilo per hour
1:15 Ligatures about kidney pedicles tied down tightly

1:44 Blood sugar 93. Rate of injection 110 mgm. per kilo per hour
2:44 Blood sugar 107. Rate of injection 147 mgm. per kilo per hour
3:48 Blood sugar 116. Rate of injection 137 mgm. per kilo per hour
4:47 Blood sugar 102. Rate of injection 149 mgm. per kilo per hour
5:48 Blood sugar 105. Rate of injection 183 mgm. per kilo per hour
7:50 Blood sugar 91. Rate of injection 197 mgm. per kilo per hour

Three such determinations were carried out together with three control animals in which kidney function was not interfered with. The results are plotted in figure 1. It is quite evident that the requirement is markedly increased after stopping kidney function. The course of these animals is in sharp constrast with that of the animals that have intact kidneys throughout. We made sure of activity of the kidneys in all control cases by injecting phenol red intravenously and recovering it later in the urine.

In the second series hepatectomy was performed with loose ligatures placed about the kidney pedicle at operation. In one case the ligature was placed around only the ureters. Kidney excretion was stopped as before, after a preliminary utilization rate determination. The results, together with those for 3 control hepatectomized animals, are charted in figure 2. The results are the same in this type of preparation as those with evisceration.

In another experiment we followed the effect of reëstablishing kidney excretion after it had been stopped for a preliminary period. Here we performed hepatectomy and placed ligatures around the ureters; these were tied snugly at the time of operation with the ends brought out through the skin of the back and tied in bows there. After a 3 hour determination of sugar requirement with kidney excretion stopped, the ligatures were released and pulled out of the body so that the ureters became free. After this phenol red was injected intravenously and it was found later in the urine, thereby proving that kidney excretion had reëstablished itself. As indicated in figure 3 the sugar requirement steadily decreases after the release of the ureters.

Conclusions and discussion. It is quite apparent that the lack of kidney function markedly increases the sugar-requirement of the eviscerated, and of the hepatectomized rabbit. In three of the animals only

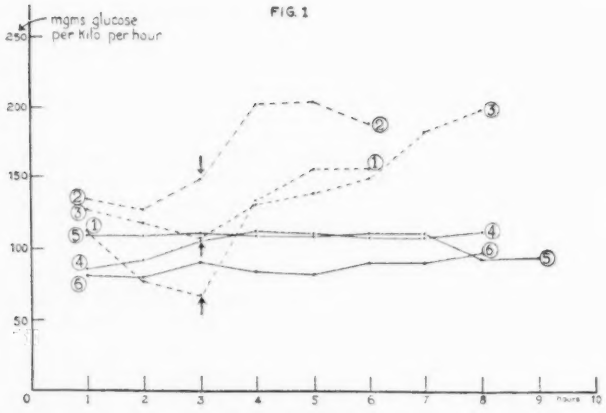

Fig. 1. The effect of ligation of the kidneys on glucose utilization rates of eviscerated rabbits. Broken line, ligated animals. Unbroken line, controls. Arrows indicate time of ligating the kidneys.

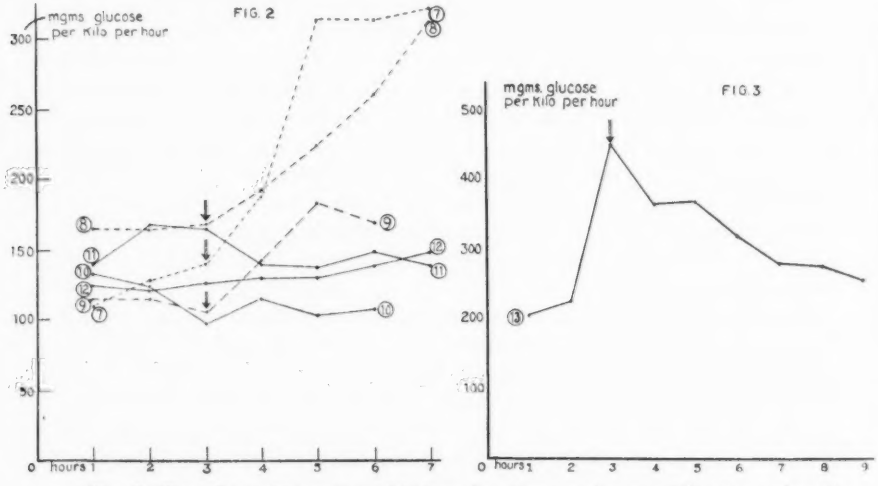

Fig. 2. The effect of stoppage of kidney function on glucose utilization rates of hepatectomized rabbits. Broken line, ligated rabbits. Unbroken line, controls. Arrows indicate time of ligation. In rabbit 7 ureters were ligated. In rabbits 8 and 9 the kidney pedicles were ligated.

Fig. 3. The effect of reëstablishing kidney function on glucose utilization rate of a hepatectomized rabbit. The ureters were ligated at the time of operation. The arrow indicates the time of release of the ligatures.

excretion was interfered with by ligating the ureters, leaving the circulation intact. These animals gave results similar to those with stoppage of the circulation through the kidneys, so that we may infer that this relationship of the kidney to glucose requirement is connected with its excretory functions. In other words we may say that the kidney excretes a substance or substances which if dammed back in the body cause a marked increase in the requirement of glucose in hepatectomized and in the eviscerated animal. What is this substance? It is not insulin since the effect is obtained in eviscerated animals in which the pancreas is removed, and similarly it can not be due to anything absorbed by the gastrointestinal tract. It might be some specific substance secreted by such an organ as the pituitary or it might be a product of metabolism of all tissues in general. If the latter should be the case, that is, that living tissues produce a substance affecting the glucose requirement of the tissues and which ordinarily is taken to the kidneys to be excreted, then this would have a bearing on conclusions which one could derive from studies of the metabolism of tissue slices after they have been out of the body for more than 2 or 3 hours. Our results indicate that the carbohydrate metabolism in patients suffering from acute and chronic kidney disease should be definitely disturbed as a result of the renal insufficiency. That this carbohydrate metabolism disturbance is not confined to hepatectomized and eviscerated animals is shown by the work of Loeper, Lemarie and Tonnet (12) who have demonstrated that in the rabbit insulin action is markedly increased by nephrectomy. Aubertin and Tresquier (13) have proved this for the dog. Villabet et al. (14) find low glycogen values in the livers and muscles of rabbits after nephrectomy, which also suggests a carbohydrate metabolism disturbance.

However it is especially in the field of metabolic research that our findings have a bearing. The reviews of Cori (15), MacLeod (16) and Peterson (1) indicate that our present day view of carbohydrate metabolism in diabetes is largely based on the results of studies on hepatectomized diabetic animals. Examination of the original papers reveals that in most of such experiments the kidneys were removed, and if they were left in, no special observation was made to make sure that the kidneys were functioning properly. This check on the kidneys must be made since after hepatectomy or evisceration the kidney is sometimes anuric.

The high glucose requirement of hepatectomized, nephrectomized diabetic animals cannot be used to support the contention that in diabetes the glucose utilization of the tissue is high. Any preparations, from which the kidneys were removed or which were noted to be anuric, must be discarded as regards estimating the glucose utilization of diabetic animals. In future, in any experiments which attempt to measure the carbohydrate

metabolism of diabetic animals in this way, particular care should be taken to see that the kidneys are functioning normally.

SUMMARY

Evidence is presented to prove that the eviscerated, and the fasted hepatectomized animal are excellent preparations for the quantitative study of the rate of sugar utilization of the remaining tissues of the body. The physiological processes of the animal—other than those directly connected with the organs removed—must be normal if one is to make this assumption.

Removal of the kidneys or stoppage of their function in such preparations leads to a marked increase in their glucose requirement.

Deductions made in the past as to the sugar utilization rate of the tissues of normal and of diabetic animals which are based on observations on hepatectomized animals without kidney function, must be reconsidered in the light of our findings.

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PRESSOR SUBSTANCE IN THE CORTEX OF THE KIDNEY

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Ever since it was found that persistent hypertension can uniformly be produced in the dog by partially obstructing the arterial blood flow to the kidneys (1), the mechanism responsible for the elevation in blood pressure has aroused much interest and speculation. Of possible significance in this connection is the finding that extracts prepared from the ischemic kidneys of such animals contain greater concentrations of pressor substances than do extracts of control kidneys (2, 3, 4). Tigerstedt and Bergmann (5) were the first to demonstrate in the normal kidney the presence of a pressor material which they called renin. Their observations were confirmed and extended by other investigators (6–8), who found that the pressor principle is thermolabile, is protein-like in nature, is limited to the renal cortex, and acts, over a prolonged time interval, either on blood vessels directly, or by way of vaso-motor nerves. We have attempted to investigate further the nature, mode and site of action of this substance.

METHOD. The pressor effect of the extracts (made as described below) was tested upon anesthetized dogs (nembutal, 25 mgm./kgm., injected intravenously) in which blood pressure was recorded directly from the femoral or the carotid artery with a mercury manometer. An oncometer and a plethysmograph, equipped with Brodie's bellows recorders, were used to study changes in the volume of the kidney and a limb, respectively.

In a number of experiments, after control records were obtained, the intestines, spleen and pancreas were removed and further readings were made. The level of the blood pressure was generally not lowered by the procedure; in fact in certain instances it rose.

The extracts were also tested upon the isolated dog's tail and the isolated dog's kidney, perfused with citrated blood or Tyrode solution. The dog's tail preparation was made in the following manner: Several hundred cubic centimeters of blood were drawn through the carotid artery, citrated, and saved for perfusion. The tail was cut off and its artery was located on the ventral surface in the mid-line and cannulated. Perfusion fluid was injected into the arterial cannula by means of a syringe, until the solution ran out of the veins freely. The cannula was then con-

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nected to the perfusing system, consisting of a pump to which was attached a 1 cc. Luer syringe, which, in turn, terminated in a two-way valve. Perfusing fluid was drawn from a reservoir and sent through a series of glass coils immersed in a water bath at 37°C. and then into the cannula. In order to insure a sensitive preparation, it was necessary to begin the perfusion no later than five minutes after amputation of the tail. The blood entering the tail eventually passed out through the open ends of the veins which had been severed at amputation. The pressure in the system was arbitrarily fixed at about 100 mm. Hg, by varying the stroke volume and rate of injection at the beginning of the experiment. Thereafter, a constant volume was delivered to the tail by the pump; hence variations in the calibre of the blood vessels were reflected in alterations in the pressure within the system, the latter being recorded on a kymograph by means of a mercury manometer and float. When the pressure level became constant, the extract to be tested was injected into the lumen of the tubing leading from the reservoir to the inflow valve of the syringe. In this way no change was produced in the pressure within the system as a result of the introduction of additional fluid.

In the case of the kidney preparation, the organ was removed from the body, and the renal artery, vein, and ureter were cannulated. The artery was connected to the perfusing system, and the blood from the vein was returned to the reservoir and oxygenated. The kidney was immersed in a water bath at 37°C. and the pressure in the perfusing system was recorded in the same manner as in the case of the tail.

Preparation of extract. In early experiments five different batches of the raw material were prepared by pressing freshly chopped pigs' kidneys, as described by Bingel and Strauss (6). The press-juice, when injected intravenously into anesthetized dogs, produced a profound, often fatal decline in blood pressure, followed, in the case of only two of the batches, by a rise of more than 10 mm. of mercury above the initial level. Since the depressor substances found in crude extracts of kidneys, as well as of other tissues, are present in the deproteinized alcohol-soluble fractions (9), the following method, suggested by Harrison, Blalock, Mason and Williams (4), was adopted for obtaining a satisfactory and potent extract, relatively free of these materials.

The tissue was cut into small pieces, placed in 95 per cent alcohol (or occasionally in ether) and allowed to stand in the ice box for a few hours. The supernatant fluid was discarded and the residue ground to a fine powder in a mortar and again stored on ice in two volumes of alcohol or ether. The addition of a few drops of hydrochloric acid to either of these solvents (two drops of concentrated HCl to 100 cc. of solution) apparently helped to produce a more potent preparation. Final extracts for injection were prepared as needed in the following manner:

The alcohol-insoluble fraction was dried in a desiccator and shaken with normal saline (5 cc. saline per gram of dry tissue) for 30 minutes. The mixture was centrifuged and the supernatant fluid (representing the alcohol insoluble portion) was saved. In this state, the extract deteriorated rapidly on standing. Table 1 indicates the disappearance of the pressor effect and the simultaneous increase in the depressor effect of a saline

extract which has been permitted to stand in the ice box for 48 hours. When kept under alcohol, however, the vaso-constrictor property was retained with somewhat diminished potency for at least three weeks.

The pressor principle could be reprecipitated from the saline extracts by means of alcohol, acetone, or 0.6 saturation with ammonium sulphate.

TABLE 1

Instability on standing of the pressor substance in neutral saline extracts of alcohol-insoluble fractions of rabbit kidney tissue

AGE OF EXTRACT	DOSE INJECTED	MAXIMUM DROP IN BLOOD PRESSURE	MAXIMUM RISE IN BLOOD PRESSURE
hours	*gm. per kgm. body weight*	*mm. Hg*	*mm. Hg*
2	0.25	35	98
24	0.4	30	38
48	0.25	60	0

TABLE 2

Pressor effect of fractions of saline extract of rabbit kidneys obtained with various agents. Fractions tested upon anesthetized dogs

AGENT USED TO EFFECT PRECIPITATION	EXTRACT	SUPERNATANT SOLUTION		PRECIPITATE REDISSOLVED	
		Dose injected (equivalent of kidney tissue)	Maximum rise of blood pressure	Dose injected (equivalent of kidney tissue)	Maximum rise of blood pressure
		grams	*mm. Hg*	*grams*	*mm. Hg*
HCl(pH 5)	R 25a	1.1	102	1.3	0
	R 25b	1.1	48*	2.6	0
Alcohol	R 11	2.0	6	2.8	22
	R 12	2.0	0	2.0	22
	R 25	1.2	0	1.3	22
Acetone	R 14	1.1	0	1.0	8
	R 26	2.3	0	2.2	44
	R 27	2.0	0	2.0	44
Ammonium sulfate (0.6 saturation)	R 9	5.5	0	3.0	32
	R 12	2.1	0	2.1	27

* After cocaine.

Several examples of each type of reaction are included in table 2. Acidification of the saline extract to pH 5 produced a precipitation of inactive material, the pressor substance being found entirely in the supernatant fluid.

Dialysis of the saline extract was carried out against tap water at ice box temperature using cellophane membranes (Du Pont No. 300). In

general, the results of Bingel and Strauss (6) were confirmed. After dialysis, the contents of the cellophane bag did not produce depressor responses. The pressor effects, however, were generally less than those produced by undialyzed control extracts prepared at the same time and stored for an equal period (table 3).

Heating the extracts in a boiling water bath for 5 minutes destroyed the pressor effect. This is in agreement with the findings of previous investigators (5, 6) who used crude saline extracts or press-juice.

Source. The concentration of the pressor material, as prepared in the manner described above and tested upon anesthetized dogs, was found to vary in different animals, being highest in the kidneys of rabbits and cats and lower in those of sheep, bulls, dogs, men and pigs. The pressor material is confined entirely to the cortex of the kidney, no pressor effect being elicited with extracts of kidney medulla, spleen or liver.

TABLE 3

Dialysis of saline extracts of rabbit kidneys. Pressor effect of the contents of the bag tested on anesthetized dogs

SAMPLE	DAYS DIALYZED	DOSE INJECTED	MAXIMUM DROP OF BLOOD PRESSURE	MAXIMUM RISE OF BLOOD PRESSURE
		gm. of dry tissue	*mm. Hg*	*mm. Hg*
R4	1	2.4	0	18
	Control not dialyzed	2.2	0	30
R27	1	2.0	0	20
	Control not dialyzed	2.0	0	52
R9	3	3.0	0	32

Type of reaction. The pressor response obtained by intravenous injection of renal extract into anesthetized animals begins immediately after injection and reaches a peak in 2 to 3 minutes. This peak is maintained for a short interval and then declines very slowly over a period of from 10 to 30 minutes. The curve can readily be differentiated from that obtained with the rapidly acting epinephrine and pituitrin, but resembles that seen after injection of tyramine. The renal pressor substance however differs from tyramine in its non-duffusibility and its insolubility in alcohol. Furthermore, a pharmacological difference between these two substances has been noted in their vasomotor effects after cocaine (10). Figure 1 is typical of the results in 10 experiments in which tyramine and renal pressor substance were tested in anesthetized animals before and after cocainization (5 mgm. cocaine hydrochloride per kgm. body weight). In every instance cocaine inhibited the pressor action of tyramine but not that of kidney substance. Similar observations have been reported recently by Williams et al. (11).

We found, as did Bingel and Strauss (6) and others (4), that repeated injection of the same extract at short intervals produces progressively diminishing effects. For this reason, when more than one sample was to be tested, at least 30 minutes were allowed between injections.

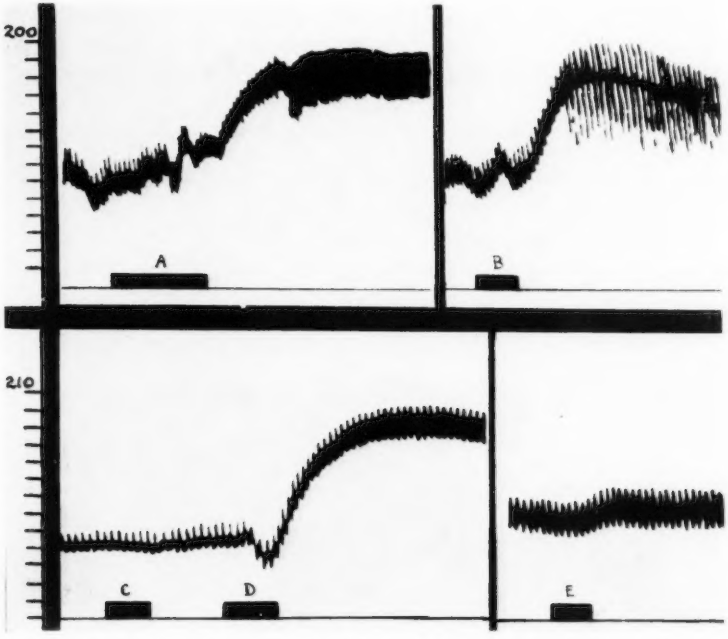

Fig. 1. Comparison between pressor effect of kidney cortex and tyramine before and after intravenous injection of cocaine. Carotid blood pressure in anesthetized dog. *A*, 7 cc. (1.4 grams) of kidney cortex. *B*, 3 cc. of tyramine, 1:10,000 dil. Between *B* and *C*, 80 mgm. cocaine hydrochloride were administered. *C*, 3 cc. of tyramine, 1:10,000 dil. *D*, 7 cc. (1.4 grams) of kidney cortex. *E*, 3 cc. of tyramine, 1:10,000 dil. Injections were made at sufficient intervals to allow for the effect of the previous administration to be lost, except in the case of cocaine which was quickly followed by tyramine (*C*) and then kidney cortex (*D*).

MODE AND SITE OF ACTION OF RENAL PRESSOR SUBSTANCE. In an attempt to investigate the site of action of the renal pressor substance, the following experiments were performed:

1. *Section of the spinal cord in the cervical region.* In two experiments, one on a dog (expt. 11) and the other on a cat (expt. 30), the effects of intravenous injections of renal pressor substance were observed before and after cutting the cord at the level of the 4th cervical vertebra. The response to the pressor material was not diminished following this procedure;

actually, it was greater than in the intact animal, a finding which is confirmatory of previous work (5, 6). The level of blood pressure after section of the cord was lower than before, which may, in part, explain the more marked response.

2. *Effect of eviscération.* In five experiments the pressor effect of renal substance, before and after removal of the intestines, the spleen, and the

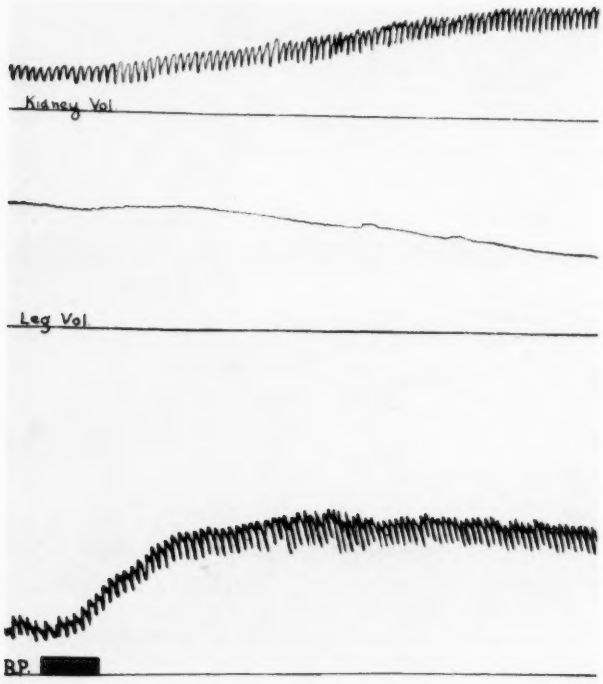

Fig. 2. Effect upon kidney volume (increase), leg volume (decrease), and systemic blood pressure (rise) of the intravenous injection of 1.5 grams of kidney cortex into the anesthetized dog.

pancreas was compared. In all instances no significant alteration in the pressor response followed this procedure.

3. *Effect upon limb and kidney volume.* In every instance in which we studied changes in limb volume, injection of renal pressor substance was followed by a decline in volume, denoting vaso-constriction coincident with the rise in blood pressure (fig. 2). In one case this was preceded by a transient rise in limb volume. On the other hand, the control extracts of

kidney medulla, or renal pressor material which had be enboiled, produced no effect.

Examination of the results obtained with the kidney oncometer reveals that in 7 out of 11 experiments, the elevation in blood pressure was accompanied by a preliminary drop in kidney volume, followed by a rise (which in most cases was of considerable magnitude (fig. 2)) beyond the previous baseline. In three instances the elevation in blood pressure was accompanied by a rise only, and in one case by a decline in kidney volume.

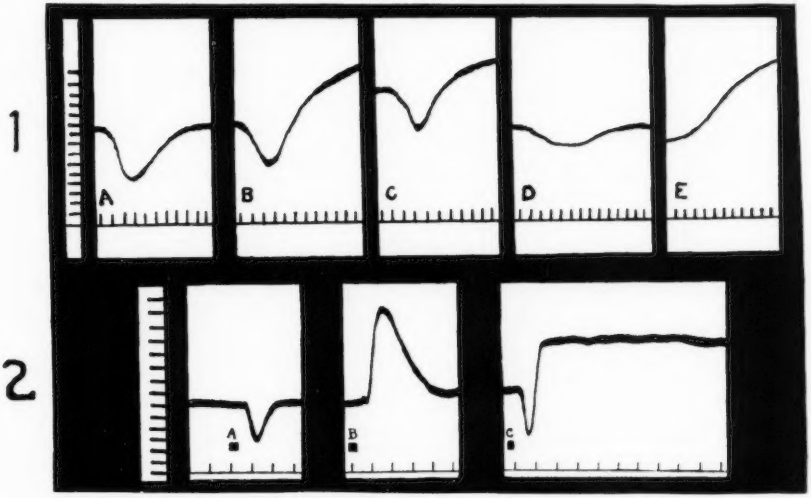

Fig. 3. Isolated dog's tail preparation perfused with citrated blood. Perfusion pressure recorded in mm. Hg. 1. Administration of kidney cortex. *A*, addition of filtrate of boiled kidney cortex (1 cc.) to perfusion fluid. *B*, *C*, and *D*, addition of same quantity (1 cc.) of untreated kidney cortex to perfusion fluid at 20 minute intervals. *E*, addition of epinephrin (1 cc.) 1:200,000 dil. to perfusing fluid. 2. Comparison of effect of 1 cc. of (A) liver, (B) spleen and (C) kidney cortex when added to perfusion fluid. Time in minutes.

Injection of extracts of spleen, liver, kidney medulla, or of heated renal cortex produced only the initial drop or else no effect at all. The above findings are in accord with those reported by Bingel and Claus (12) who used a crude extract.

4. *Effect upon the isolated, perfused dog's tail.* The results obtained on the perfused, amputated dog's tail varied, depending upon the freshness of the preparation and the nature of the perfusing fluid. When the tail was perfused with Tyrode's solution, or when some delay occurred in

making the preparation, extracts of all tissues produced more or less marked vaso-constriction. In these instances the pressor action was greatest with spleen and liver, intermediate with renal medulla and least with renal cortex. Frequently only depressor effects were observed with extracts of kidney cortex.

When citrated dog's blood was used as the perfusing fluid in the fresh tail preparation, the results were different. Extracts containing the renal pressor material produced a prolonged and well sustained pressor effect sometimes with and sometimes without a transient initial depressor action (fig. 3). The pressor response lasted in some instances for more than 30 minutes. Splenic extracts usually induced a depressor effect or a sharp but transient pressor response. Extracts of liver showed either no effect or a transient depressor response (fig. 3). Boiling the extract abolished the pressor action of renal cortex (fig. 3), but not that of spleen.

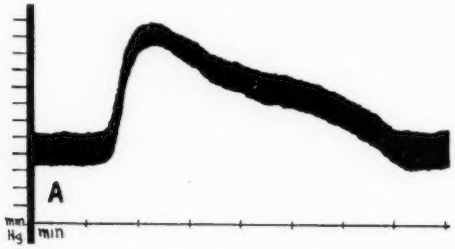

Fig. 4. Effect of kidney cortex upon isolated dog's kidney perfused with citrated blood. Perfusion pressure recorded in mm. Hg. A, addition of 0.3 gram kidney cortex to perfusion fluid.

Repeated injections, at short intervals, of equal doses of a potent renal pressor substance were followed by progressively diminishing vaso-constrictor effects. In this respect the tail preparation gave a response resembling that observed with repeated injections in the intact animal. The explanation for the diminishing effect is not known at present. That the vessels still retained the ability to constrict in response to a suitable stimulus was demonstrated by the pressor effect elicited with epinephrin (fig. 3).

5. *Effect upon the isolated dog's kidney:* In six experiments in which the isolated kidney was perfused with citrated blood, the injection of renal pressor material resulted in an elevation of pressure in the system, sometimes with and sometimes without a slight preliminary drop (fig. 4). This was not elicited by the boiled extract.

Discussion. Bingel and Strauss (6), Hartwich and Hessel (7), and others have found greater pressor effects in autolyzed than in crude renal

extracts. The presence of tyramine in products of protein autolysis and the known ability of the kidney to transform tyrosine into tyramine (13) suggest that the action of autolyzed kidney extracts may, in part, be due to the presence of tyramine. This has recently been demonstrated by Williams et al. (11), who also noted the failure of cocaine to inhibit the effect of renal pressor substance.

The pressor material in the alcohol-insoluble fractions used in this investigation is definitely not tyramine. Like the crude saline extracts (the "renin" of Tigerstedt) it is thermolabile, nondiffusible, insoluble in alcohol, acetone, ether, and 0.6 saturated ammonium sulfate solution, and is found only in the cortex of the kidney. Whether this pressor material and renin are identical cannot be stated on the basis of the present evidence.

It would appear from the above data that the renal pressor substance does not act by way of the higher centers, but rather that it has a local pressor effect upon the blood vessel itself. Since the eviscerated and the intact animal are equally responsive, it is apparent that the pressor principle does not achieve its effect predominantly by acting upon the splanchnic area. Further evidence supporting the view that the systemic vascular bed plays a rôle in the rise of blood pressure is supplied by the constant finding of vasoconstriction of blood vessels in the perfused dog's tail on addition of the renal substance to the perfusing fluid.

The response of the kidney deserves further elaboration, for this organ generally increased in volume as the systemic blood pressure rose. Richards and Plant (14) have presented evidence to show that in the perfused kidney, injection of very small quantities of epinephrin (less than 0.0002 mgm.) causes a rise in perfusion pressure, indicating vasoconstriction of blood vessels, and somewhat later a swelling of the kidney. A plausible explanation offered by these investigators was that epinephrin in very small quantities affects principally the efferent vessels, causing glomerular distention and consequently an increase in kidney volume. The findings with renal pressor substance may possibly be interpreted in a similar light, the enlargement or shrinkage of the kidney being entirely dependent upon whether the effect is predominantly on the efferent vessels or on the generalized renal vascular bed.

There is abundant clinical and experimental evidence that persistent hypertension may be associated with a variety of injuries to the kidney. Studies on hypertensive patients and on dogs with experimental hypertension following renal ischemia have led investigators to the conclusion that the elevation in blood pressure is caused by an increase in peripheral resistance resulting from a generalized arteriolar hypertonus (15, 16). This increased arteriolar tonus is not mediated by way of the sympathetic nervous system either in the experimental animal (16) or in man (15) (with the possible exception of acute nephritis (17)). Moreover, the

evidence indicates that in animals with hypertension due to renal ischemia the stimulus arising within the kidney must be chemical in nature, rather than nervous (18).

An hypothesis explaining the known facts in renal hypertension would, therefore, postulate a chemical stimulus of renal origin, exerting a generalized pressor effect on the blood vessels, and being independent of the central nervous system. The renal pressor substance investigated in the present work appears to possess properties which are compatible with and lend support to this view.

SUMMARY

1. A pressor substance with protein-like properties is present in saline extracts of alcohol-insoluble fractions of the cortical portion of fresh kidneys.

2. It differs from tyramine in its chemical and pharmacological properties.

3. It acts on both splanchnic and peripheral vessels, independently of the central nervous system.

The authors wish to acknowledge with thanks the technical assistance of Joseph Marrus, Edward Weissbard and Fannie A. Senior.

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THE EFFECT OF THE ADMINISTRATION OF SOME CARBOHYDRATE DERIVATIVES ON THE HYPOGLYCEMIC SYMPTOMS OF THE HEPATECTOMIZED DOG

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In 1922 Mann and Magath found that complete removal of the liver in dogs resulted in a steady fall of the blood sugar level. When the blood sugar reached a very low level typical symptoms appeared, and ultimately convulsions and death supervened. Administration of glucose was effective in curing or preventing these symptoms, and in prolonging the life of the animals for many hours. The administration of only four substances other than glucose, viz: mannose, maltose, dextrin and glycogen, was found to provide any relief from these hypoglycemic symptoms (Bollman and Mann, 1936). Bollman and Mann believe that these substances relieve the symptoms of hypoglycemia because they are rapidly converted to glucose in the blood. More recently Griffiths and Waters (1936) have shown that the administration of fructose may prevent the onset of symptoms by acting as a glucose sparer. The liverless rabbit resembles the hepatectomized dog in requiring the frequent administration of glucose, or substances closely related to glucose, to prevent the onset of hypoglycemic convulsions (Drury and Salter, 1934).

The administration of insulin to intact animals induces the appearance of hypoglycemic symptoms which resemble those following removal of the liver, and the capacity of many substances to replace glucose in preventing or curing these symptoms has been investigated. Although the interpretation of the results of such experiments are complicated by the possibility that the substance under investigation is converted to glucose in the liver, the negative nature of the majority of these results emphasizes the peculiar specificity of glucose in alleviating the symptoms of hypoglycemia.

The demonstration by Embden, Deuticke and Kraft (1933) and Meyerhof (1933) that hexose diphosphoric acid, dihydroxyacetone phosphoric acid, (Meyerhof and Lohmann, 1934a) alpha glycero-phosphoric acid and pyruvic acid are all intermediary metabolites, in the formation of lactic acid from glycogen in muscle, suggested the possibility that these substances

[1] Work carried out during the tenure of a Beit Memorial Fellowship.

might share with glucose the capacity to relieve the hypoglycemic symptoms of the hepatectomized dog. The presence of ascorbic acid in many tissues of the body (Bessey and King, 1933) and the possible importance of this reactive substance in metabolism, suggested the interest of determining its influence on the course of the symptoms.

EXPERIMENTAL. Dogs weighing 6 to 12 kgm. were used in these experiments. The liver was removed by the method of Markowitz, Yater and Burrows (1933).

In some experiments, at the first sign of convulsive twitchings the substance to be tested was rapidly injected intravenously, in amount calculated to be equivalent to 2–4 grams of glucose. When this amount was ineffective in preventing the onset of vigorous convulsions, a second and sometimes a third injection of an equal amount of material was given. A negative result was then controlled by the intravenous injection of 1 to 2 grams of glucose which should, and usually did, cause obvious relief of the condition in one or two minutes.

It is possible that a substance can act as a glucose-sparer in general metabolism, and thus be capable of delaying the onset of symptoms in the hepatectomized animal, but be of no value in alleviating the symptoms once they are established. Therefore in some experiments the substance under test was continuously infused into the saphenous vein by means of an automatic pump, in amount calculated to be equivalent to about 0.25 gram of glucose/kgm. body/hour. The infusion was always begun as soon as the animal had recovered from the operative anesthesia. At the onset of convulsions a rapid injection of an amount of material equivalent to about 2 grams of glucose, was given in addition, and a negative result controlled by the injection of glucose. The glucose equivalent of an equimolecular mixture of glycerophosphoric acid and pyruvic acid was calculated on the basis that one molecule of glycerophosphate plus one molecule of pyruvate were equivalent to one molecule of glucose.

Dihydroxyacetone phosphate was prepared by two methods. For the first experiment it was synthesized by the method of Kiessling (1934). The calcium salt was not further purified, but immediately converted into the sodium salt. As Kiessling pointed out, the product is sufficiently pure for most biological experiments and we certainly detected no evidence of toxicity in the hepatectomized dog. For the second experiment the ester was obtained from hexose diphosphate by the enzymic procedure of Meyerhof and Lohmann (1934b).

Injections were made into the jugular or saphenous veins, and acids were administered as their sodium salts.

RESULTS. In our experiments the animal usually showed hypoglycemic symptoms culminating in convulsions in one to two hours after the operation of hepatectomy had been completed. Infusion of any of the sub-

stances investigated apparently had no effect on the time at which symptoms appeared, and the injection of large amounts had no effect on the symptoms once they had commenced. The results are summarized in table 1.

DISCUSSION. Our negative results do not necessarily constitute evidence against the belief that the substances tested are intermediate products in the metabolism of glucose. This is clear from a consideration of the nature, and possible mechanism, of the symptoms which accompany hypoglycemia.

TABLE 1

NUMBER OF EXPERIMENT	SUBSTANCE INJECTED	METHOD OF ADMINISTRATION	TOTAL AMOUNT ADMINISTERED, GLUCOSE EQUIVALENT	INFLUENCE ON SYMPTOMS
			grams	
4	Hexose diphosphoric acid	Periodic injection	4.5	None
8	Hexose diphosphoric acid	Continuous infusion	4.9	None
11	Hexose diphosphoric acid	Continuous infusion followed by rapid injection	6.0	None
14	Dihydroxyacetone phosphoric acid	Continuous infusion	4.5	None
15	Dihydroxyacetone phosphoric acid	Continuous infusion	2.0	None
5	α-Glycerophosphoric acid plus pyruvic acid	Periodic injection	5.0	None
6	α-Glycerophosphoric acid plus pyruvic acid	Periodic injection	5.0	None
7	α-Glycerophosphoric acid plus pyruvic acid	Continuous infusion	4.1	None
9	Ascorbic acid	Periodic injection	2.0	None
10	Ascorbic acid	Periodic injection	2.0	None

That the symptoms which follow the diminution of the blood sugar level in the hepatectomized animal are due ultimately to a lack of available glucose in the blood seems certain, for the prompt and complete relief of the condition by injection of a small amount of glucose is such a striking and uniform phenomenon. It should be mentioned that when the blood sugar of a diabetic animal is lowered, either by the removal of the liver or by the administration of insulin, typical hypoglycemic symptoms may occur when the blood sugar is at a level well above its normal value. In these cases, however, the symptoms are relieved by glucose administration, and must therefore be due to an absolute or relative lack of glucose.

The central nervous system must be involved in the manifestation of symptoms of hypoglycemia, for a denervated limb does not react under

conditions which cause a similar intact limb to exhibit hypoglycemic convulsive movements (Best, Hoet and Marks, 1926). The spinal cat shows no convulsive signs even in the severest insulin hypoglycemia (Brooks, 1934), while according to Drabkin and Shilkret (1927), water starved dogs do not undergo typical convulsions during severe insulin hypoglycemia, but lapse into coma which may be followed by death. Griffiths and Waters (1936) have suggested that an end-organ might be stimulated by a lack of glucose, and thus originate a reflex culminating in convulsions.

The actual mechanism whereby glucose lack results in hypoglycemic symptoms is obscure. The two chief possibilities are: *a*, the tissues require an adequate "glucose environment" for normal functioning in much the same way that isolated muscle tissue requires the presence of particular inorganic ions in its environmental medium; *b*, glucose undergoes a metabolic reaction which supplies energy in a specific and unique manner; without energy derived from this source the tissues cannot function properly. Although the first possibility cannot be entirely ruled out, it seems improbable, on general grounds, that glucose is so vital to the tissues because of its physical properties alone. The alternative explanation seems more probable. The peculiar specificity of glucose may result from the fact that its oxidation may, by means of "coupled reactions" (cf. Green, 1937), initiate a whole series of chemical processes, the maintenance of which is necessary for the normal functioning of the tissues. Glucose might be irreplaceable because the process of oxidation of any other substance (e.g., one formed from glucose by oxidation or glycolysis) could not be coupled in the specific manner.

The possibility must also be considered that glucose is required for different purposes in different tissues, and that no other single substance can replace glucose in all these reactions. It is clear, therefore, that the failure of any substance to relieve hypoglycemic symptoms in the hepatectomized dog cannot be considered as evidence against the view, entertained as the result of other experiments, that the substance plays an intermediary rôle in the metabolism of glucose.

A number of the substances investigated by us are phosphoric esters, and this fact alone may have been sufficient cause for the negative results, as the ability of such esters to diffuse through living tissues is possibly extremely small (Rothschild, 1929).

SUMMARY

1. Administration of the following substances has been found to exert no influence on the hypoglycemic symptoms which follow hepatectomy in the dog: hexose diphosphoric acid, dihydroxyacetone phosphoric acid, a mixture of alpha glycerophosphoric acid and pyruvic acid, ascorbic acid.

2. The nature and possible mechanism of production of hypoglycemic

symptoms is discussed and the suggestion is made that inability to cure or prevent hypoglycemic symptoms does not constitute evidence against the idea, entertained as the result of other experiments, that a particular substance plays an intermediary rôle in carbohydrate metabolism, either glycolytic or oxidative.

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AN EXPERIMENTAL ANALYSIS OF HUMAN LOCOMOTION

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Of the group who have studied walking, running and related movements (Weber, Braune and Fischer, Hill, Fenn, Kreezer and Glanville, Knoll, Schmith, Schwartz, and their associates), only Hill and Fenn have attempted to correlate movements with muscular activity. In this present study the activity of certain key muscles of the lower limb, as shown by action currents and deformation, was recorded in conjunction with the movements of locomotion in order to determine experimentally the actual relations between muscular action and movement in human locomotion.[1]

APPARATUS. The subjects moved on a treadmill fitted with roller bearings and thoroughly lubricated to reduce friction. The recording apparatus permitted the subjects complete freedom of movement. The subjects reported that the conditions closely approximated normal walking and running.

Most of the material was recorded on a kymograph especially designed for recording skilled movements. The horizontal drum eliminated the gravity factor in the recording. The drum speed was 10 to 12 cm. p. s. (cf. Stetson and Bouman, 1935).

Muscle deformation was recorded pneumatically. Some features of this technique have been discussed by Stetson and Hudgins (1930) and by Stetson and Bouman (1935). The pneumodeik eliminates radial distortion. The tubes leading to the applicators on the subject were taped together into a cable and reënforced internally with a coiled wire spring. Accidental vibrations (echoes, etc.) in these connecting tubes were damped out with capillaries 2 cm. long. These do not obscure the significant characteristics of the primary waves. The records show no systematic artifacts (cf. figs. 4 and 5). Deformation of the muscle was picked up with light cork-boss applicators equipped with condom rubber diaphragms. These applicators were taped to a small area directly over the muscle, with the cork boss forced into the muscle by the rubber diaphragm. By actual calibration the lag of this system was found to be between 11 and 12 ms., approximately 1 ms. for each foot of tubing. The footfall was

[1] Dr. C. V. Hudgins gave much valuable advice and assisted with some of the recording. Mr. J. M. Snodgrass did the oscillographic recording.

recorded by means of a section of light-walled rubber tubing closed at one end; this end was placed under the transverse arch of the foot. The pneumatic systems were closed at atmospheric pressure.

Movement was recorded with a thread-and-rubber-band system, described by Stetson and Bouman (1935), which gives a close approximation to the actual movement. One line recorded the movement of the knee; this was fastened just above the patella. The other line recorded the movement of the foot (heel) and was attached to a small wire loop in the rear of the running shoe. The recorded movement was 0.145 of the actual movement. By calibration the lag of this system was found to equal approximately that of the pneumatic system. Since the records were read only to 5 ms. no correction was made for the lag.

TABLE 1

Records taken under controlled conditions

SUBJECT	TOTAL NUMBER OF RECORDS	TYPE OF MOVEMENT		
		1. Oscillation	2. Walk	3. Run
II	11	4	3	4
III	14	4	4	6
IV	10	3	3	4
V	15	4	5	6
VI	11	3	3	5
VII	10	3	3	4
VIII	8	2	3	3
IX	6	2	2	2
X	10	3	3	4
XI	9	3	3	3

A synchronous timer and base line marker was developed to facilitate reading records with five pneumatic tracings and two movement tracings. This wrote into the records a straight base line for each pneumatic tracing. The base-line markers were connected to two electric markers which interrupted the base lines at 0.1 sec. intervals. Time was supplied by a synchronous motor geared to 10 rev. p. s.

The condom-rubber diaphragms used on the applicators were the only variable elements in the recording system. These diaphragms could be depended upon for about three days before they aged sufficiently to rupture under pressure. In order that the same diaphragms might be used on all ten subjects, the series of records were made within three days. The list of records taken is given in table 1. Only one diaphragm had to be replaced during this time. The pneumatic systems were so stable that the markers did not have to be reset during a run.

Kymographic material was substantiated with simultaneous oscillo-

graphic recording of action currents. These joint records showed clearly that the deformations, as recorded kymographically, were accompanied by action currents. However, correlating this material was rather complicated. To avoid this and to obtain records which would show the relation betwen the movement tracing and the tracings of the deformation and the action current, the three tracings were recorded simultaneously in the oscillograph, a nine-element Westinghouse model. One of the records

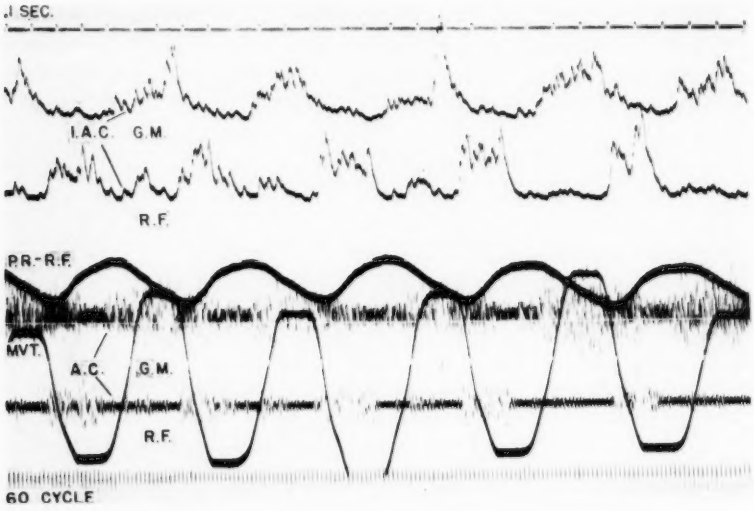

Fig. 1. Relation of deformation, action current and movement in oscillations of the pendent lower limb.

A.C.—action current; *I.A.C.*—integrated action current; *P.R.*—pneumatic recording of deformation; *Mvt.*—movement; *R.F.*—rectus femoris; *G.M.*—glutaeus maximus.

The averages of readings from this record are given in the first column of table 2. These averages are shown graphically in figure 2. Calibration of the pneumatic system showed that the deformation developed slowly and receded rapidly. The glutaeus maximus action current is over-amplified. Both action currents show some leakage.

is shown in fig. 1. On this particular record there was an average interval of 34 ms. between the beginning of the action current and the beginning of the deformation. The computed lag in the pneumatic system was between 11 and 12 ms. This means a net interval of 22 ms. Three other records taken with the same arrangement showed net intervals of 14 ms., 19 ms., and 23 ms. (cf. table 2). The beginning of the deformation always followed the beginning of the action current. Since the interval was fairly constant there seems to be a definite relation between them.

This interval was also noted in the joint records mentioned above. Whatever the components of this interval may be, it was evident that there was no danger of placing the muscle action too early in the movement cycle if the beginning of the deformation were used in relating the muscle action to the movement. It was evident also that the pneumatic systems were recording some definite resultant of muscular contraction, probably the active tension of the muscle.

PROCEDURE. The muscles selected were the glutaeus maximus and the rectus femoris for the thigh, and the vastus medialis and the gastrocnemius for the leg. These muscles are representatives of the flexor and extensor groups, and judging from other movements such representatives will indicate the action of these antagonistic muscle groups. The ileopsoas

TABLE 2

Averages of readings from oscillograms

Series K 272 K8A18

RECORD NUMBER	29E	30F	31AG	31BH
Number of readings	5	5	5	4
Duration of cycle	532 ms.	535 ms.	507 ms.	486 ms.
Events in cycle:				
1. Backstroke begins	−248	−236	−231	−227
2. Action current begins	−208	−191	−198	−201
3. Deformation begins	−186	−177	−179	−178
4. Backstroke ends	−101	−88	−85	−77
5. Action current ends	−11	−5	−31	−13
6. Beatstroke begins	000*	000	000	000
7. Deformation ends	95	98	93	111
8. Beatstroke ends	187	196	191	164

* Beginning of beatstroke is zero point. Minus sign indicates time before zero point (cf. fig. 2).

is not accessible either for deformation applicator or for action-current electrodes.

In the action-current recording the electrodes were placed on either side of the deformation applicator in order that the results might be comparable.

The recordings were in the nature of samples. Unless the subject chose to signify when the movement was running smoothly, the recording apparatus was started when the movement was running smoothly. The subject was free to choose his own rate in walking and running. In limb oscillation the subject was instructed to swing the limb back and forth at a brisk rate. Sometimes during the recording the subject was told to increase the rate in order to get a breakdown of the movement.

FINDINGS. Part 1: *Oscillations of the pendent lower limb.* In locomotion the lower limb must execute compound reciprocal movements and also support the body. To find how the muscles of the thigh produce the

movement cycles when this postural factor is eliminated, simple to-and-fro movements (oscillations) of the limb were studied. The limb was moved back and forth while hanging from the pelvis. In this "type" movement (limb oscillation; cf. movement tracings in figs. 3 A, 4 A and 5), the movement cycles showed long periods of uniform velocity both in the forward and backward strokes. This indicates that the limb was moving as a result of momentum. This momentum phase in the movements makes them "ballistic," or *thrown*, movements. These same, relatively long, momentum phases have been noted in other high-speed movements (Stetson and Bouman, 1935; Peters and Wenborne, 1935–36; Wachholder, 1928). The maximum rate was between 2 and 2.5 per second. An attempt to drive the limb faster caused at first a sacrifice of amplitude, and finally a breakdown of the movement (cf. fig. 5). This breakdown consists of a fixation which interrupts the movement cycle when the antagonistic driving muscles

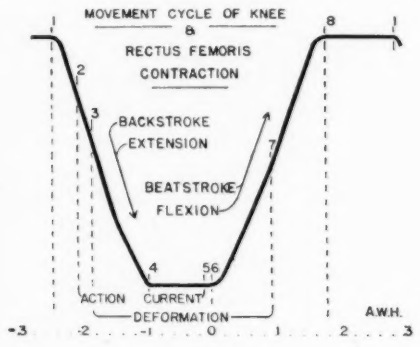

Fig. 2

are contracted simultaneously (Stetson and Bouman, 1935). Figure 4 A shows a kymograph recording of the driving contractions of the rectus femoris and of the glutaeus maximus in the movement cycle of limb oscillation. Figure 3 A shows a diagram based on averages of readings from this record. As recorded, the contractions of each muscle occupied about a third of the movement cycle. Each contraction ended when the ballistic phase of the stroke that it produced began. There was no overlapping of antagonistic contractions.

In order to determine more accurately the place of the muscular impulses in the cycles of the oscillating movements, two aspects of muscular action, action current and deformation of the muscle, were recorded with the movement tracing in the oscillograph. One of the oscillograms is shown in figure 1. A diagram based on averages of readings from this oscillogram is shown in fig. 2. Table 2 gives the averages of readings from this and other similar ocillograms. The rectus femoris is instrumental in flexing

the hip-joint and causing the forward stroke of the thigh (indicated by a rise in the movement tracing). Figures 1 and 2 and table 2 show that the rectus femoris was not active during the whole of the stroke that it produced; on the contrary, its activity occurred chiefly during the stroke in the opposite direction (backstroke) which preceded the stroke that it caused (beatstroke). The movement while reversing should undoubtedly trace a hairpin curve. The truncated reversal, with its shoulders, is a frictional artifact. However, since the movement at that stage was too slight to record, the conditions under which the muscle was developing tension were essentially isometric.

In an intact subject the length of the muscle depends on the movement of the limb. During the reversal of direction in the movement cycle the rectus femoris is in an essentially isometric condition. The slight deviation from the isometric condition is both positive and negative so that it averages isometric. However, reference to figures 1 and 2 and to table 2 shows that the beginning of activity in the rectus femoris, as recorded by the action current and by the deformation, occurred well before the beginning of the beatstroke and also well before the end of the backstroke. During the backstroke the extension of the hip-joint was lengthening the rectus femoris. The muscle was developing tension under what might be called pliometric, lengthening, conditions. The action current stopped before the beatstroke began. However, the deformation of the rectus femoris continued during part of the beatstroke under conditions in which the muscle was actually shortening, as a result of its tension, and drawing the limb with it. The linear condition of a muscle exerting tension while shortening might be called miometric. The three conditions, pliometric, isometric and miometric, cover all the possible conditions with respect to length under which a muscle could develop and maintain tension. In this case the rectus femoris stopped the extension and began the flexion of the hip-joint in each movement cycle with a single period of more-than-normal tension, which began under pliometric conditions, continued under isometric conditions and stopped under miometric conditions at the onset of the ballistic phase of the stroke. It is obvious that the term "contraction," when used to describe a muscular state, must mean this period of more-than-normal tension, and that it can have no reference to the shortening of the muscle.

In these reciprocal ballistic movements the driving contractions begin while the movement is lengthening the muscle, continue while the conditions are essentially isometric, and only about the last fourth of the driving contractions occur while the muscles are shortening. Furthermore, reference to figures 1 and 2 and to table 2 shows that the action current, representing the active change in the muscle, ceased either before the stroke began or before it had progressed far.

The constant off-phase relation between the action current, with the

deformation, and the movement indicates that these are three related manifestations of a single process, the process by which muscles produce movements. The action current accompanies the active changes in the muscle which produce the deformation. The deformation may shorten the muscle; but when the muscle cannot shorten freely, as in the contractions driving reciprocal ballistic movements, the tension developed exerts force

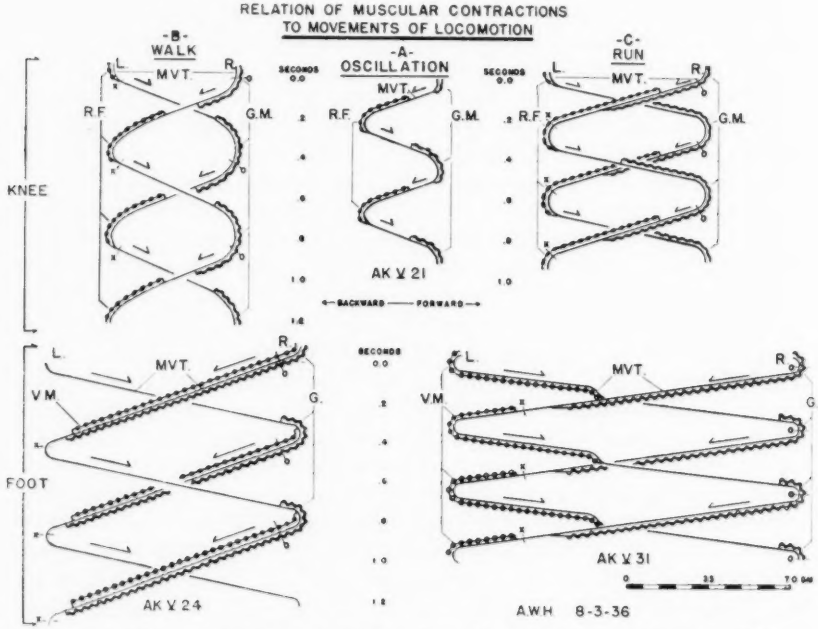

Fig. 3. *Mvt.*—movement; *R.*—right; *L.*—left; *R.F.*—rectus femoris; *G.M.*—glutaeus maximus; *V.M.*—vastus medialis; *G.*—gastrocnemius; *O*—footfall; *X*—toe leaves tread.

The vertical time line was used in order to preserve the normal hip-knee-foot relation. Contractions were recorded pneumatically. The movements and contractions were actually recorded only from the right limb. Since the runner's feet were in contact with the tread only about 70 per cent of each cycle, the length of his stride was really slightly over 2 m.

on the limb. The reciprocal ballistic movement is the simplest of movement cycles since it involves two strokes in opposite directions. The movement cycle is driven by muscular contractions, which produce action currents and deformation of the muscle. The force from the period of tension development figures in the movement cycle as an impulse. In figures 1 and 2 we have a contraction of the rectus femoris similar to the

contractions driving the movements of walking and running (cf. fig. 3). The action current begins during the backstroke. As the muscular tension developing under pliometric conditions takes hold of the limb the stroke is decelerated. The action current and the deformation continue until the movement of the limb is reversed. The action current stops before the beatstroke starts, or at least while the muscle is under essentially

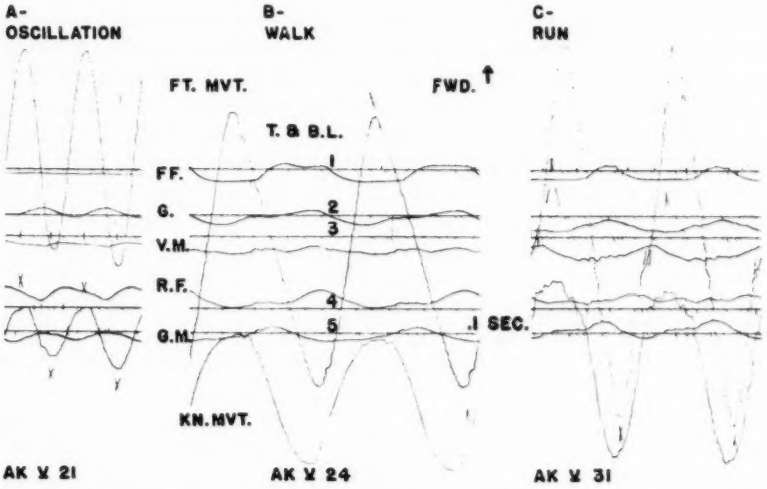

Fig. 4. Kymographic recording of movements and of representative muscular contractions.

Ft. Mvt.—thread-and-rubber-band tracing of foot (heel) movement; *Kn. Mvt.*—same for knee; *T.* and *B.L.*, *1,2,3,4,5.*—time and base lines; *FF.*—footfall, pneumatic recording; *G.*—gastrocnemius, same; *V.M.*—vastus medialis, same; *R.F.*—rectus femoris, same; *G.M.*—glutaeus maximus, same. Excerpts of records; original size 30 by 64 cm.

Averages of readings from these records were used as a basis for figure 3. The fact that the muscular contractions are not in phase with the movements is clearly shown. Muscular contractions were recorded with cork-boss applicators. Calibration of the pneumatic recording system showed that the deformation accompanying the contraction developed slowly (rising line) and receded rapidly (sinking line). The beginning and end of the deformation show clearly.

isometric conditions. The deformation continues during the period of acceleration of the limb and drops out as the ballistic phase of the stroke begins (fig. 3 A). It seems certain that in movements of this sort there is little or no loss of muscular energy through viscosity. The type of movement (ballistic or tense), the rate of movement and the amplitude of movement depend on the incidence of the contraction, the duration and intensity of the contraction and on the activity of the antagonistic muscles.

The efficiency of a muscle in producing a movement decreases when the muscle shortens during the development of tension and when the antagonistic muscles oppose the action of the agonist. Since the contraction is often out of phase with the actual movement, it is necessary to determine the phase relation experimentally in a given movement at varying rates before discussing the relation.

Part 2: *Oscillations of the pendent limb as a key to the driving and postural contractions of walking and running.* Pendent oscillations free from postural contractions are the simplest reciprocal movements of the lower limb (figs. 3 A and 4 A). Since the rate of the movement cycle was close to maximum with neither stroke accented, the forward and backward strokes were equal in duration and extent. The contractions of the rectus femoris and of the glutaeus maximus occupied about a third of each move-

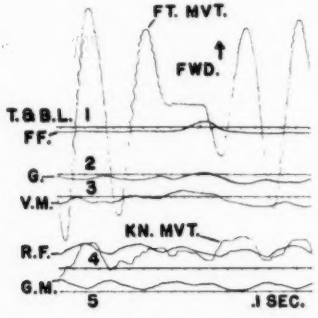

Fig. 5. Breakdown of movement cycle in oscillation of pendent lower limb resulting from effort to increase rate of movement. (For explanation of abbreviations see fig. 4.)

The foot movement shows clearly the decrease of amplitude followed by fixation which accompanies an attempt to speed up the movement cycle. Maximum rate about 2.5 p. s.

ment cycle. Each occurred just before the ballistic stroke that it produced and ended as the ballistic phase of the stroke began. The movement of the foot (heel) paralleled that of the knee.

Walking. The functional requirements of walking altered the simple reciprocal pendent movements of the thigh and leg without changing their ballistic nature (figs. 3 B and 4 B). Even the foot striking and leaving the tread of the mill did not alter perceptibly the foot movement. The forward and backward strokes of the knee remained equal in duration and extent, but the forward stroke of the foot was considerably more rapid than the backward. The knee reached the extent of its forward stroke slightly after the foot, but it reached the extent of its backward stroke more than 0.1 sec. before the toe did and considerably before (0.077 sec., average) the foot left the tread.

The action of the rectus femoris was strikingly similar in limb oscillation and in walking (compare figs. 3 A and B). The contraction of the glutaeus maximus in walking began at the same relative point as in limb oscillation, but the diagram (fig. 3 B) shows that the contraction continued while the limb was moving backward. However, the kymograph tracing from the muscle (fig. 4 B) ascended, reached a plateau, and then from this plateau, after perhaps a slight drop, ascended to a second plateau before dropping to the base line. The end of the driving contraction was apparently obscured by a postural contraction. The glutaeus maximus had insured the stroke of its limb backward to the tread as the contralateral rectus femoris was reaching the culmination of its contraction. The rectus femoris pulling on an unsupported pelvis would have rotated it forward. This was prevented by the postural contraction of the glutaeus maximus, and probably by other muscles about the pelvis. If the rectus femoris had been opposed by its ipsilateral glutaeus maximus a breakdown would have occurred in the movement; but the fixation of the pelvis by the contralateral glutaeus maximus would cause no breakdown. This action of the glutaeus maximus, therefore, by immobilizing the pelvis, forced the rectus femoris to raise the limb forward rather than to incline the trunk forward. The two contractions of the glutaeus maximus were probably separate and distinct and, as such, should be represented separately on the diagram (fig. 3 B). The functional requirements of walking did not alter the fundamental relation noted above between the driving contractions and the ballistic phases of the movement cycles.

In contrast to running (see below) none of the muscles studied seemed to have as its primary function driving the epicycloidal movement of the leg which swung freely from the moving knee. The vastus medialis preserved the extension of the knee during the backward stroke. This was a postural, rather than a driving contraction and occurred under substantially isometric conditions. The gastrocnemius acted to prevent the flexion of the foot beyond a certain point. Its contraction was carried on under pliometric or essentially isometric conditions. Since the gastrocnemius contraction continued after the knee started its forward stroke and ended before the toe left the tread its function must have been largely postural.

The functional requirements of walking caused the inclusion of certain postural contractions with the driving contractions. Like the driving contractions, the postural contractions were developed against forces that tended to lengthen the muscles. The postural contraction of the glutaeus maximus was the only one, of the contractions recorded, that occurred while the muscle was shortening. However, the disposition of the glutaeus maximus about the hip-joint would make this shortening so slight that even this postural contraction took place under substantially isometric conditions. With this possible exception, the contractions of the postural

muscles were carried on under pliometric or isometric conditions. The function of the postural contractions was to immobilize the pelvis and the segments of the backward moving limb so as to provide a firm base from which the opposite limb could be thrown by the driving contractions of its rectus femoris and associated muscles.

Running. The movements of running are also ballistic (figs. 3 C and 4 C). The increase in the rate of movement changed the movements. With this subject, the foot (heel) took approximately the same time for its forward and backward strokes, but the forward stroke of the knee was more rapid than the backward stroke. The knee reached the limit of its forward stroke considerably before the heel struck (average 76 ms.), but the heel reached the limit of its forward stroke at about the same time the heel struck. Neither foot of the subject was in contact with the tread during about 30 per cent of each movement cycle.

The contraction of the rectus femoris in running resembles closely that of walking and of limb oscillation. The contraction of the glutaeus maximus is similar to that of walking. Apparently the same postural contraction following the driving contraction was necessary in this case as was noted above. The two should probably be separated on the diagram (fig. 3 C).

The function of the contraction of the vastus medialis in the running cycle was quite unlike that noted in walking. The contraction was not used to stabilize the knee during the stroke backward to the tread, but to extend the knee during the return (forward) stroke. The contraction caused a change in the path of the foot movement with some acceleration. This acceleration was accompanied by a deceleration of the knee. The result was not as much the acceleration of the foot as the straightening of the knee in order that the heel might strike the tread rather than the toe. The contraction, which was executed about a ballistically moving pivot, occurred under pliometric and isometric conditions.

As in walking, the gastrocnemius contracted to prevent the flexion of the foot beyond a certain point. The contraction occurred under pliometric and isometric conditions and ceased before the foot left the tread. The gastrocnemius helped to stabilize the leg. The glutaeus maximus on the same side fixated the pelvis. This provided a firm base from which the rectus femoris and vastus medialis of the opposite limb could throw that thigh and leg forward.

Discussion. Richer (1895), on the basis of experimental work, differentiated between rapid, ballistic movements and slow, moving fixations, i.e., tense movements. Evidence confirming the ballistic nature of rapid movement may be found in the papers of Wachholder (1928), Peters and Wenborne (1935–36, 1936) and Kreezer and Glanville (1937). Stetson and Bouman (1935) have extended Richer's study and confirmed the analysis. The present study shows that in locomotion the limb movements

are ballistic and that the concomitant postural movements are likewise produced by unopposed impulses.

However, there is a maximum rate for free, ballistic movements, for if the rate of a to-and-fro movement with ballistic strokes is increased there comes a stage at which cocontractions inevitably occur and the movement breaks down into a fixation or a tense movement. In the reciprocal ballistic movement the force necessary to decelerate, reverse and throw the limb increases with the rate of movement. Since the mass of the limb remains the same the momentum increases with the velocity. But the rate of tension development (intensity of contraction) is definitely limited. This sets a limit to the velocity of the ballistic stroke. It also sets a limit to the duration of the contraction which must degenerate the force of the stroke. When both strokes of the reciprocal movement reach the maximum velocity the rate of movement can only be increased by decreasing the length of the ballistic strokes. Since the duration of the driving contractions remains constant as the duration of the movement cycle shortens, there is a point at which the contractions begin to overlap. At this point the cycle breaks down.

Gould and Dye (1932) state that human responses "must be carried out with extreme slowness and through relatively short distances . . . in order to . . . approach the isometric twitch both in nature and efficiency." On the contrary it is the rapid, ballistic movement which is efficient. Such movements should be coördinated so that the momentum of one ballistic stroke provides the posture for the next. Slow movements, which are usually moving fixations, should be avoided. Human locomotion has a relatively high efficiency because tense movements in which muscles work against each other are avoided, because the rate of repeated movements is kept below the rate at which the amplitude of the long, ballistic strokes must be sacrificed, and because these ballistic movements are driven by substantially isometric contractions.

SUMMARY

1. In limb oscillation, walking and running the movement cycles of the thigh and leg show long, ballistic strokes, one forward and one backward. Between these ballistic strokes the movement of the limb is decelerated, reversed and accelerated by a muscular contraction (a period of more-than-normal tension as shown by action currents and deformation). During the contraction of the agonist the antagonist is relaxed, and during the ballistic phase of the stroke both muscles are relaxed.

2. The contraction of the agonist begins during the backstroke while the limb is lengthening the muscle (pliometric conditions). It continues under substantially isometric conditions and then, in the early part of the beatstroke, under shortening (miometric) conditions. Tension de-

velopment (action current and hardening of the muscle) occurs under pliometric and substantially isometric conditions. Deformation continues under miometric conditions during the acceleration of the limb as the tension is transformed into kinetic energy.

3. Posture and movement depend on the same musculature and upon muscular tension developed under similar conditions. Postural contractions counteract forces that tend to lengthen certain muscles during the movement cycle. Tension is, therefore, developed under pliometric and essentially isometric conditions. Posture is the support from which the agonist drives the movement. This support is not rigid; it may be the momentum of a segment of the body, or it may be a concomitant stroke or series of strokes developing momentarily and disappearing as the agonist relaxes.

4. The rate at which free ballistic movements can be repeated is limited. At a certain rate cocontractions are inevitable and the movement cycle breaks down into a fixation. This rate in limb oscillation is between 2 and 2.5 cycles p. s.

5. The relatively high efficiency of human locomotion depends on the use of long, ballistic strokes, upon the avoidance of cocontractions and fixations, and upon the development of tension for both driving and postural contractions under pliometric and substantially isometric conditions.

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EVIDENCE FOR THE HORMONAL NATURE OF THE OXYTOCIC PRINCIPLE OF THE HYPOPHYSIS[1]

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Since the discovery by Dale (1909) of oxytocic activity in extracts of the posterior lobe, much effort has been expended in attempts to determine whether or not this organ may be considered an endocrine gland which elaborates an oxytocic hormone. The arguments for and against such an hypothesis are reviewed exhaustively by Van Dyke (1936), who concludes that the favourable evidence is at best suggestive while evidence to the contrary is not lacking. Much the same view is expressed by Reynolds (1937).

In the experiments reported in this paper electrical stimulation of the pituitary stalk has produced definite and at times enormous augmentation of uterine motility. Control experiments have, in our opinion, demonstrated clearly that these effects are due to the release of an oxytocic hormone from the hypophysis.

METHODS. Rabbits 2 to 80 hours *post partum* were used in most of the experiments, since preliminary observations had revealed too great a variation in the response of nonpregnant animals. In a typical preparation, the animal was anesthetized with chloralose and urethane (1 per cent chloralose in 10 per cent urethane, 5 cc. per kgm. intravenously). Following tracheotomy and, usually, section of the vagi in the neck, the uterus was exposed by midline incision, the tubal end of one horn ligated and a cannula of suitable size inserted through the corresponding cervix. The uterus was filled with warm Ringer's solution and connected through a transmitting system to a stiff tambour. By adding solution through a side tube the pressure in the uterus was adjusted to a level of about 1 cm. of Ringer's during relaxation. With active contractions pressures of 55 cm. and over were recorded. The side tube was usually kept closed during recording—thus obtaining virtually isometric contractions.

This method of recording uterine activity, as with most others, is not

[1] The expenses of this investigation were partially defrayed by a grant from the National Academy of Arts and Sciences. We are grateful also to Dr. H. A. Charipper, of New York University, for his kindness in making available some of the experimental material.

entirely satisfactory. Only a synchronous contraction of the whole horn gives a large deflection of the tambour. If one part contracts while another relaxes there may be no indication of activity on the record. For this reason some direct observations of the exteriorized uterus were made while records were being taken. The uterus was covered with a petri dish to afford some protection from loss of heat and moisture. In the *post partum* uterus conditions were excellent for recording. The typical activity consisted in a contraction starting at the tubal end and rapidly (in about 15 sec.) passing over the whole horn. An example of fallacious recording was evident when epinephrine was injected. On the record one large contraction was shown, followed by apparent inhibition. By direct observation it was seen that during this period of apparent inhibition the uterus was by no means quiescent but was occupied by several rings of stationary contraction which seemed to block the propagation of synchronized contraction. Between the rings were zones of relaxation which prevented a general rise of pressure in the uterine cavity.

In the nonpregnant uterus contractions seemed to start anywhere although most frequently at the uterine flexure or at the cervical or tubal ends. The contractions were poorly propagated, the net effect being an irregular record rarely showing either complete relaxation or synchronized contraction.

Stimulation. The skull was trephined over the bregma and oriented in the stereotaxic instrument ingeniously improvised by Dr. A. J. Derbyshire for the experiments of Haterius and Derbyshire (1937) on ovulation in the rabbit. This permitted the insertion of bipolar electrodes with fair precision to any desired position in the brain stem. The electrodes were insulated to the tips, leaving an area of exposure of each electrode of no more than 0.25 $mm.^2$. The interelectrode distance was about 0.5 mm. The stimulating current was supplied by a Harvard inductorium driven by two dry cells. The vibrator was weighted to give a frequency of 6 to 8 per second. A secondary coil distance of 6 to 7 cm. was usually used although excellent responses were obtained with a coil distance of 9 cm.

At first, stimuli were applied in the midline in the region of the hypothalamus (supra-optic area) found by Haterius and Derbyshire to be effective in producing ovulation in the rabbit, but it was soon found that more consistent results were obtained directly over the stalk of the pituitary.

Additional preparation of animals prior to the initial stimulation was varied, as indicated in table 1. In many cases the vagi, cervical sympathetics and cardiac depressors were cut. In one instance spinal transection was made at level 7–8 T, together with bilateral interruption of the splanchnic nerves at their point of entry into the abdominal cavity. In a few cases, the cord was cut between the 4th and 5th thoracic vertebrae.

In others the nervous system was kept intact until an initial response had been obtained. Finally, in blocking the response, a direct current ranging in strength from 0.2 to 1 milliampere was applied directly to the pituitary

TABLE 1

Uterine response to stimulation of the pituitary stalk

ANIMAL	HOURS POST PARTUM	PREPARATION OF ANIMAL	COMMENTS
		A. Summary of cases showing positive responses	
1	60	Intact	Response repeated after spinal transection (4–5 T)
2	60	Vagotomy	Response repeated after spinal transection (7–8 T)
3	60	Intact	Response repeated after spinal transection (7–8 T); abolished by burning of stalk
4	72–80	Vagotomy	Response repeated after spinal transection (7–8 T) and splanchnotomy
5	48	Vagotomy	Response repeated after spinal transection (7–8 T)
6	30	Vagotomy Splanchnotomy Spinal transection (7–8 T)	Died before burning of stalk
7	20	Vagotomy	Response abolished by burning stalk
8	16	Vagotomy	Response abolished by burning stalk
9	12	Intact	Response abolished by burning stalk
10	16	Intact	Response not obtained after spinal transection (7–8 T). *Post mortem:* mechanical damage to stalk by electrodes in initial attempt at stimulation.
11	36	Vagotomy	Response abolished by burning stalk
12	12	Vagotomy Spinal section (4–5 T)	Response abolished by burning stalk
		B. Summary of cases showing no response	
13	48	Vagotomy	Uterus failed to respond to intravenous Pitocin. *Post mortem:* large hematoma at cervical end of uterine horn
14	24	Intact	*Post mortem:* point of stimulation 3 mm. behind stalk
15	55	Vagotomy Spinal section (4–5 T)	*Post mortem:* point of stimulation 1 mm. lateral to and 3 mm. behind stalk

stalk 1 or 2 mm. below the level of stimulation. All operative procedures were verified at autopsy.

RESULTS. Before stimulation was attempted the spontaneous activity of the uterus was recorded for one to two hours. In the *post partum* rabbit this activity consisted typically in synchronous contractions occurring at

intervals of one to one and a half minutes (fig. 1 *et seq*). In some cases the frequency and amplitude of the spontaneous activity tended to diminish during the control period, in others the level of activity was well maintained. A typical response in the intact animal is shown in figure 1 and in figure 5*A*, and is seen to consist in a marked increase in frequency and, usually, in amplitude. Moreover, the less active the uterus prior to stimulation, the more striking the response. This is exemplified in figure 3, in which, after unsuccessful attempts at burning the stalk, and the lapse of two hours, an enormous response was obtained upon subsequent stimulation.

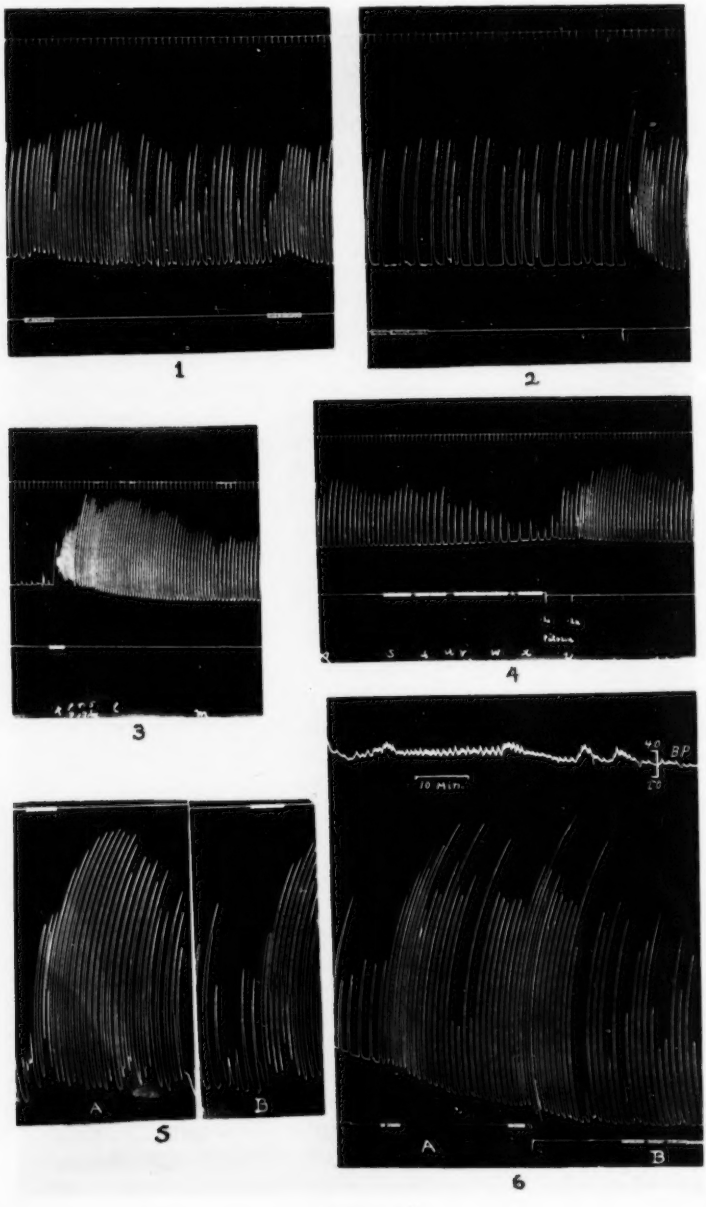
A response was best elicited when the electrodes were placed in, or 1 to 2 mm. above, the infundibular foramen; this was usually about 17 mm. from the surface of the exposed cortex. The area in which a response could be evoked seemed to be quite limited; in many instances stimulation as close as 2 mm. to one side of the stalk or behind it proved ineffective.

Abolition of the response was accomplished readily by producing an electrolytic lesion in the stalk. This was done by moving the electrodes down 1 to 2 mm. Often they were then deflected forward by the posterior edge of the foramen, and thus were applied directly to the stalk. With the electrodes in the right place burning for 10 minutes sufficed to block any subsequent response. In one instance (rabbit 7) considerable difficulty was encountered in abolishing the response, and burning was repeated at several levels in the stalk and in the gland before a complete block was obtained. Examination *post mortem* revealed that the initial burns were about 1 mm. away from the stalk. Figures 3 and 4 are graphic records of the uterine responses of this rabbit. This animal served to demonstrate that the mechanical effects of moving the electrodes in and through the excitable region may not impair the excitability. In one instance, however (rabbit 10), we have reason to believe that mechanical damage by the electrodes blocked the response.

Rabbit 8 is representative of the intact animals in which a response was obtained which subsequently was abolished by burning the pituitary stalk, and in figure 1 may be seen the graphic record of the responses elicited by stimulation, first 1 mm. above the stalk and then directly in the infundibular foramen. After a 10 minute burn at a strength of 0.8 milliampere repeated stimulation failed to evoke further responses, although 0.2 unit of Pitocin intravenously produced a typical effect (fig. 2).

In this instance, and this has been our experience throughout, direct stimulation of the gland, at least after destruction of the stalk, evoked no response. Of some significance too is the observation that following destruction of the stalk uterine activity diminished progressively despite repeated stimulation.

A response following spinal transection is shown in figure 5, in which *A*



Figs. 1-6

represents the response of the intact animal (rabbit 1), 60 hours *post partum*, and *B* shows the response after spinal transection between the 4th and 5th thoracic vertebrae. This level is sufficiently high to exclude the possibility of an influence of the splanchnic nerve on the response.

In two animals (rabbits 3 and 12) a complete experiment was performed, i.e., in the single animal the response was obtained after interruption of all possible nervous pathways, and was then blocked by burning the pituitary stalk. Figure 6 is the graphic record of uterine activity in rabbit 12, 12 hours *post partum*, in which, in addition to preliminary vagotomy and spinal transection at level 4–5 T, blood pressure recording was taken from femoral artery. Excellent responses were elicited, as shown in figure 6, *A*, from stimulation immediately above the stalk. Following a 12 minute burn just within the foramen further stimulation, repeated at several levels, proved ineffective (fig. 6, *B*). Examination *post mortem* revealed the stalk cleanly burned through and the gland intact. Spinal section involved the 5th thoracic spinal roots.

In figures 2, 3 and 4 may be seen the striking similarity of the responses obtained by electrical stimulation and the intravenous injection of Pitocin, even to the duration of responses which persisted for as long as fifty minutes or even an hour. From a comparison of the responses one might infer that the pituitary could expel into the blood as much as 0.5 unit of oxytocin at one time.

The injection of epinephrine (1 cc. of 1/50,000) gave a very different result. One forceful contraction was followed by an apparent inhibition which lasted for three or four minutes and then the normal activity was resumed.

Table 1 gives a summary of all the experiments on *post partum* rabbits except those in which the animal died before stimulation was applied. In

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Fig. 1. Rabbit 8; 16 hours *post partum* showing rhythmic activity. Increase in frequency and amplitude in response to stimulation of pituitary stalk. Points of stimulation indicated by signal magnet on lower line. One minute time intervals.

Fig. 2. Same animal. Failure of response after burning pituitary stalk. Stimulation applied above stalk and in hypothalamus; 0.2 unit Pitocin given intravenously at termination of experiment. Note slowing of rhythm after burning of stalk.

Fig. 3. Rabbit 7; 20 hours *post partum*. Response of a quiescent uterus to stimulation of pituitary stalk following an unsuccessful attempt at interrupting stalk.

Fig. 4. Same animal. Failure of response after burning of stalk, despite repeated stimulation. Final response obtained by 0.2 unit Pitocin intravenously.

Fig. 5. Rabbit 1; 60 hours *post partum*. *A*—uterine response in intact animal. *B*—response following spinal transection at level 4–5 T. Points of stimulation indicated in upper line.

Fig. 6. Rabbit 12; 12 hours *post partum*. *A*—responses following spinal transection at level 4–5 T and vagotomy. *B*—abolition of response by burning pituitary stalk. Upper tracing blood pressure in femoral artery; lower tracing signal magnet.

it are indicated the various procedures by which the possible nervous connections to the uterus were severed. Cases in which no response was obtained are included separately. In the light of the *post mortem* findings they seem to be as instructive as the successful experiments.

Virgin rabbits were tried at various times in the course of this work. The results are inconclusive but certain observations are presented for what they may be worth. In general, the records of uterine activity showed rapid irregular fluctuations which were interpreted as asynchronous activity of different parts of the uterus. In four out of ten animals a distinctive response was obtained on electrical stimulation of the infundibular region resembling that following injection of Pitocin. In other rabbits the uterus was insensitive to Pitocin, a condition not uncommon, apparently, in unanesthetized nonpregnant rabbits (Reynolds, 1930). In one such animal electrical stimulation gave a definite response which persisted after destruction of the hypophysis but was abolished by spinal transection between the 5th and 6th thoracic vertebrae. The injection of epinephrine gave a response similar to the one following electrical stimulation.

Discussion. The foregoing experiments, we believe, clearly demonstrate that, upon suitable stimulation of the hypophyseal complex, uterine motility in the *post partum* rabbit is greatly increased in frequency and amplitude. That the exciting factor in the uterine response emanates from the pituitary seems likely in view of the facts that 1, the optimal area for electrical stimulation lies immediately above or in the pituitary stalk and is well localized, and 2, that an adequate electrolytic lesion of the stalk abolishes the response. Following effective burning, further stimulation above the stalk or elsewhere in the hypothalamus proves ineffective. It seems highly probable that the factor released through such electrical stimulation is the oxytocic principle of the pituitary, since it exerts an effect on the *post partum* uterus strikingly similar to that caused by the administration of Pitocin. That it is not due to the liberation of epinephrine is demonstrated by the fact that it may be obtained after section of the splanchnic nerves or after high spinal section. Epinephrine, moreover, in the *post partum* rabbit produces a uterine response very different from that resulting either from Pitocin or from stimulation of the infundibular region.

On the basis of these experiments it is, of course, impossible to specify the portion of the hypophysis from which the hormone is released, but for the time being it seems safe to assume that it originates in the pars neuralis. It is apparent, moreover, that the release of oxytocin is to some extent under nervous control, a point which is of interest in any consideration of the reflex control of uterine activity—a phase of reproductive physiology concerning which little is known. The afferent paths involved and the

possible relations to the sex hormones are subjects for speculation as yet, but it is believed that the method employed in these experiments may be expected to yield more information concerning the important problems of uterine motility and of parturition.

SUMMARY

In twelve rabbits, 2 to 80 hours *post partum*, electrical stimulation in the region of the infundibular stalk produced a definite increase in uterine activity, characterized by increased frequency and amplitude of contractions. This response resembled closely that obtained by injection of Pitocin and differed radically from the effects of epinephrine. The response persisted after spinal transection, after section of the splanchnic nerves and after vagotomy, but was effectively blocked by electrolytic lesions placed directly in the stalk of the pituitary body.

On the basis of these observations it is believed that the pituitary complex secretes the oxytocic principle and that the latter need no longer be considered as merely a pharmacological principle but may properly be classed as a hormone of the pituitary gland.

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THE EFFECT OF CORTIN ON THE ELECTROLYTE CHANGES IN CAT MUSCLE DURING STIMULATION AND RECOVERY

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Fenn and Cobb (1936) showed that the stimulation of rat skeletal muscle through its motor nerve results in a loss of potassium from inside the fiber and an equivalent gain in sodium, a gain of sodium chloride into the extracellular spaces and a gain of water, partly extracellular and partly cellular. These changes are largely reversed during recovery. The same changes in general were found to occur as the result of voluntary contractions as well (Fenn, 1937) in cat muscle during stimulation with the additional observations that calcium, magnesium and phosphorus change little (Fenn et al., 1938). Malorney and Netter (1936) confirmed the sodium changes in rabbit muscles.

The secretion of the adrenal cortex seems to be concerned in some way with the functioning of the motor unit. Associated with a deficiency in the cortical hormone there are marked changes in plasma electrolytes, an increase in plasma potassium, and a decrease in plasma sodium and chloride (Truszkowski and Zwemer, 1936, and several other investigators in adrenal physiology). There appears to be little change in plasma calcium. Myasthenia develops along with marked hemoconcentration. Injection of cortin into animals without adrenals or into normal animals results in the converse changes in plasma electrolytes (Hartman, 1937; Harrop and Thorn, 1937).

The experiments reported here were undertaken to determine the effects of plasma electrolyte changes induced by injection of cortin, KCl, and $CaCl_2$ on the electrolyte exchanges in skeletal muscle during stimulation. The results show that cortin has a slight depressing effect on potassium loss and water gain during stimulation. This effect is similar to that resulting from the intravenous injection of $CaCl_2$ during stimulation, while the injection of KCl results in an increase in the amount of potassium lost and water taken up by the muscle.

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METHODS. Cats were anesthetized lightly by intraperitoneal dial Ciba. A tracheal cannula was inserted as routine procedure. Each gastrocnemius muscle was bared and completely separated from the soleus and surrounding tissues with care not to interfere with the blood supply. The tendons were cut and connected to isometric recording levers. The sciatic nerves of both legs were bared and sectioned, the gastrocnemius branch of the nerve was slipped over platinum electrodes incased in narrow, shellacked rubber tubing. The nerve was stimulated with condenser-like discharges at a rate of 660 a minute, using maximal shocks. Such a frequency is well within the range which gives maximal changes in rats (Fenn and Cobb, 1936). The nerves were stimulated for thirty minutes except in one series where longer stimulation times were desired. In several animals blood pressure was measured directly by a carotid cannula.

After stimulation a carotid was opened and the system drained of all the blood that would flow out, a sample being taken for plasma analysis. Each muscle was removed, cleaned of all surrounding connective tissue, dried on filter paper and one gram samples taken for analyses. All analyses could be made on one muscle. Dry weights were determined and the muscle samples dry-ashed overnight in a muffle

TABLE 1

Electrolyte changes in cat muscle during activity

(Millimols or cubic centimeters per 100 grams dry weight)

SUBSTANCE	NUMBER OF ANIMALS	AVERAGE RESTING VALUE	AVERAGE CHANGE WITH STIMULATION
		mM. or cc.	*mM. or cc.*
K	15	40.2 ± 1.3	−7.2 ±1.95
H_2O	14	242.5 ±12	+32.0 ±8.0
Na	15	7.81 ± 0.9	+6.97 ±1.0
Cl	15	5.54 ± 1.0	+2.25 ±0.8
Ca	25	0.45 ± 0.10	+0.20 ±0.08
Mg	25	2.01 ± 0.25	−0.62 ±0.7

A plus sign in column 4 indicates a gain during activity, and a minus sign a loss. Figures given are averages for all muscles analysed.

furnace. Potassium was determined by Kramer and Tisdall method (1921), sodium by the method of Butler and Tuthill (1931), with some modifications from Salit (1932). Calcium was precipitated as oxalate and titrated (Halverson and Bergheim, 1917), with some washing modifications as suggested by Wang (1935). Magnesium from the filtrate was precipitated as phosphate and determined by method of Fiske and Subbarow (1929). The chlorides were determined according to method of Van Slyke and Sendroy (1923).

RESULTS. *Changes in muscles during stimulation.* The data presented in table 1 give the average electrolyte composition of the gastrocnemius muscle of the cat in millimols (or cubic centimeters water) per 100 grams dry weight (column 3), and the changes resulting from thirty minutes of stimulation (column 4). The essential changes, completely confirming Fenn et al. (1938), are a loss of potassium and a gain of sodium, chloride and water. There is a slight but significant gain of calcium and a loss of

magnesium. Since there appears to be a gain in extracellular water some magnesium must have been lost from inside the cells. At most, however, these changes in Ca and Mg are small.

Using the chloride space as a measure of extracellular water approximately 14.5 cc. of the water gained by the muscle passed into the cells proper. Of significance is the observation that the amount of sodium gained is greater than the chloride, the excess presumably exchanging with the potassium. Such a finding is similar to that of Fenn and Cobb (1936), working with rats. However, the molar concentrations of sodium and potassium are only approximately equivalent at best, as shown in the table. Further evidence for exchange is offered by data in table 2, in which it is shown that the gain in sodium varies with that of the potassium lost rather than with chloride or water changes.

TABLE 2

Relation between electrolyte changes and time of stimulation

(Millimols or cubic centimeters per 100 grams dry weight)

NUMBER OF ANIMALS	TIME OF STIMULATION	CHANGE WITH STIMULATION			
		K	Na	Cl	H_2O
	min.	*mM.*	*mM.*	*mM.*	*cc.*
4	15	−2.24	+2.97	+1.82	+15.0
15	30	−7.2	+6.97	+2.25	+32.0
4	45	−5.5	+7.1	+2.96	+35.0
4	90	−8.2	Lost	+2.92	+10.2
6	150	−12.4	+8.3	+1.82	−5.0
6	180	−12.2	+11.4	+1.0	−8.0

As table 2 further shows, prolongation of the period of stimulation results in an increase in the amount of K lost. After quite long periods the muscles show an actual loss of water rather than the usual gain. There may be dehydration of other tissues resulting in removal of water from the active muscles (see Fenn and Cobb, 1936).

The electrolyte changes during stimulation are almost completely reversed in a three hour recovery period as is shown by the data from the normal controls in table 3. Each recovery value is an average of four muscles from four experiments. The change on stimulation then represents an average of the twelve muscles from these animals which were not allowed to recover from the stimulation. The muscles from each side were stimulated simultaneously for thirty minutes by electrical shocks from the same stimulator, one muscle immediately tied off and sampled, the other one left with circulation intact for the desired recovery period. The latter muscle was then sampled. The values in the table present

the difference between those analyses and the resting average as given in column 3 of table 1.

Injection of KCl, $CaCl_2$, and cortin. Potassium chloride (concentration 1 per cent) was injected into a small mesenteric vein at the rate of 0.65 cc. per minute for the thirty minute stimulation period. The average rise in plasma K was approximately 0.184 mM per 100 cc. of plasma. The injected K disappeared from the plasma quite rapidly. The loss of K from the plasma appeared to be more rapid than that observed in cats by Thaler (1935), but somewhat slower than reported by Zwemer and Truszkowski

TABLE 3

Changes in potassium in skeletal muscle during stimulation and subsequent recovery

(Millimols per 100 grams dry weight)

TREATMENT	AVERAGE DURING STIMULATION FOR 30 MINUTES	SUBSEQUENT RECOVERY		
		1 hour	2 hours	3 hours
	mM.	*mM.*	*mM.*	*mM.*
Normal, control	−7.2	−5.7	−2.9	−2.2
Control with injected NaCl	−7.3	−5.9	−2.7	−2.4
Injected KCl	−8.3	−7.5	−1.7	−0.2
		−6.9	−2.3	−1.1
		−7.3	−3.2	−1.0
Injected $CaCl_2$	−5.1	−3.2	−1.2	−0.7
		−3.7	−1.5	−1.0
		−3.9	−1.8	−2.0
Cortin	−4.7	−3.2	−1.0	−1.0
		−3.5	−1.8	−0.6
		−4.0	−1.95	−0.5

Recovery figures for experimental muscles are single experiments. All others are averages of several muscles. See text for full explanation.

(1936). Injection of KCl at a faster rate had marked effects on the heart and blood pressure and in some cases was toxic to the animal. Injection of 1 per cent $CaCl_2$ at same rate as the KCl injection resulted in a plasma Ca change of 0.086 mM per 100 cc. of plasma. Such injections had no appreciable effects on the tension developed by the muscles.

In the experiments with cortin each animal was injected with daily doses of cortin for three days preceding the actual stimulation experiment. Each dose contained 4 to 6 maintenance units of the cortical hormone (Hartman cat maintenance unit). Such treatment lowers plasma K and

increases plasma Na and Cl. Nine cats were used in each group of experiments.

In table 3 the recovery values are actual values of the individual experiments, the values for the stimulated muscles are averages of all the stimulated muscles in that particular group of experiments, nine in each case. The figures express the difference between the actual analysis and the average resting value taken from table 1.

The injection of KCl resulted in a small increase in the amount of potassium lost during stimulation. Any effect on recovery is certainly not very marked. Table 3 also shows that injection of isotonic sodium chloride solution during the stimulation period had no significant effect on the electrolyte changes as compared to normal control animals. Injections of $CaCl_2$ showed smaller effects than the KCl, resulting in a depression of K loss during the stimulation of the muscles. The cortin-treated animals showed results very similar to these $CaCl_2$-treated animals. Corresponding changes were observed in the amount of water gained by the muscles.

Experiments carried out on muscles other than the gastrocnemius indicated that resting muscle electrolytes are not altered significantly by any of the injections used in these experiments.

At most the effects resulting from the injection of salts and cortin are not very large. The work done by all the muscles appeared to be approximately the same under all of these experimental conditions.

Discussion. A more crucial type of experiment would be a study of adrenalectomized animals with and without injected cortin. So far such experiments have not proved feasible. The smallness of the effects when large doses of cortin are injected into normal animals indicates that cortin has little direct effect on the work a muscle can do. The cortin effect might be on some more central part of the motor unit. It remains to be shown that there is a relationship between muscular energy release and electrolyte relationships. Suggestions do arise from the fact that the longer a muscle is stimulated the greater the electrolyte change but these may simply be concomitant phenomena. Under such conditions cortin might have an effect on the contractile mechanism without necessarily having significant effects on ionic shifts.

SUMMARY

During stimulation cat skeletal muscle loses about 20 per cent of the potassium in the fibre (potassium exchanging with sodium of the plasma), loses magnesium, gains water along with sodium chloride and small amounts of calcium. These findings completely confirm the published observations of Fenn et al. (1938).

After two and one-half hours of stimulation muscles show a much greater loss of potassium with an approximately equivalent gain of sodium than

do muscles stimulated for periods of thirty minutes or less. Water may actually pass out of the muscles during such stimulation. During recovery these changes are almost completely reversed.

Injection of KCl intravenously increased the loss of potassium and the gain of water during activity; $CaCl_2$ and cortin had depressing effects on the electrolyte changes.

Neither the changing of the plasma K or Ca concentration nor the injection of cortin have significant effects on the recovery of the muscles subsequent to stimulation.

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THE BLOOD SUPPLY OF VARIOUS SKIN AREAS AS ESTIMATED BY THE PHOTOELECTRIC PLETHYSMOGRAPH[1]

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Information on the skin circulation has been supplied in the past by observations of skin color and temperature, by inspection of limited skin areas, by limited plethysmography, by measurements of skin conductivity, by oscillometry of ear arteries. The application of the mechanical plethysmograph has been necessarily limited to such areas as the ears, fingers and toes in which the circulatory changes are not confused by the participation of vascular beds in deeper tissues such as the muscles. It is possible that the ears, fingers and toes show vascular reactions unlike those in other skin areas. Such differences become increasingly probable when one considers the distribution of arterio-venous anastomosis in the skin.

The development of the photoelectric plethysmograph (1) offered the opportunity to apply the principle of plethysmography to the study of the circulation in various skin areas hitherto unexplored in plethysmographic studies. This paper details data which have been thus obtained on the skin circulation in healthy adult male subjects at rest. The data represent an attempt at estimating the arterial blood supply of various skin areas. They are, therefore, related to problems of heat dissipation by the skin, to the significance of skin temperature measurements, and to the study of vascular reactivity in various skin areas.

METHOD. Advantage is taken of the fact that the absorption of light by a transilluminated tissue varies with its blood content, to detect vascular changes with the photoelectric cell (fig. 1). One uses as an illumination source, a pencil flashlight bulb carried on a metal plunger in the metal sleeve attached to the photocell housing. The open end of the sleeve is brought into light contact with the skin so that light reflected from the skin surface passes back into the sleeve while light entering the skin scatters and so transilluminates the skin. The resulting variations

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in the photoelectric currents are recorded on the electrocardiograph after suitable amplication with a simple 3-stage amplifier (employing the 112-A tube). It is to be noted that only light which has passed through the skin reaches the photocell. This point is important as movements of the skin surface produce exaggerated effects on the photocell if light reflected from the skin surface reaches the cell. (One may take advantage of this fact to record the skin pulsations produced by the larger arteries. In this case, the plethysmograph is placed about 1 cm. above the skin surface so that only part of the skin surface under the photocell is brilliantly illuminated. The size of the illuminated area under the photocell will pulsate with the arterial pulse thus effecting corresponding pulsatile variations in the photocell current.) The intensity of the light reflected back from the skin is also a function of the blood content. This has apparently

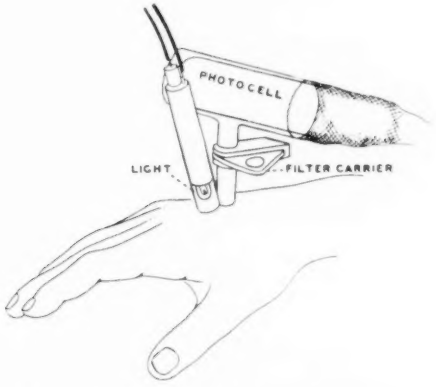

Fig. 1. The photoelectric plethysmograph in position over the skin of the hand

been used by Turner (2). The plethysmograph is carried by rack and pinion on a heavy stand for exploration of the skin of the arm, leg, hand and foot. In applying the plethysmograph to the skin of the face and forehead, it is necessary to mount it on a counter-weighted head strap due to movements of the head with the heart beat and with respiration. Adequate flexibility in alignment is provided by ball and socket joints. This arrangement is sufficiently comfortable for observation periods up to an hour in length. Although this form of the plethysmograph may be used on the ear and the data on the ear in table 4 were so obtained, another arrangement is more easily applied and is less subject to errors from movement of plethysmograph with respect to skin. In this alternate arrangement the light source is placed on one side of the ear and the photoelectric cell on the other side. The light affecting the photocell passes through

the thickness of the ear. The instrument is here suspended as before from a head strap.

Quantitation of the plethysmogram is provided for in terms of arbitrary units by recording on the plethysmogram (fig. 2) the deflection resulting from inserting a thin sheet of glass between the photocell and the skin surface. The glass sheet (called filter in the figure and hereafter) is mounted in a swinging plate attached to the photocell housing. The filter is in position to absorb light only when calibration is desired. It is otherwise swung out of line with the light path during the actual recording of the plethysmogram. The variations in the latter due to the volume pulse or to slower and larger changes in blood content are compared with the deflection resulting from the insertion of the filter and calculated in filter units by dividing the excursion in the plethysmogram due to variation in blood content by the deflection due to insertion of the filter. Thus, in figure 2, the filter deflection is 7 mm and the amplitude of the volume pulse is 5 to 6 mm. or equivalent to 0.8 filter unit.

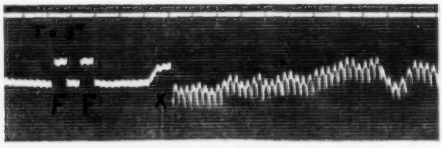

Fig. 2. Photoelectric plethysmogram of forehead illustrating method of quantitating the plethysmogram in arbitrary units with the aid of a filter, inserted at *F*. String shunt resistance increased at *X*.

The problem of the actual blood equivalent of the filter is a complex one. The sources of error in photoelectric plethysmography discussed below bear on the question. An empirical approach is provided by the data obtained by Turner, Burch and Sodeman (2) with their highly sensitive mechanical plethysmograph on the finger tip. From their table 1, one obtains an average volume pulse in the finger tip at heart level of 2.8 cu. mm. or approximately 0.06 per cent of the soft tissue volume. Under similar conditions of room temperature and humidity, the average amplitude of the photoelectrically recorded volume pulse in the finger tip at heart level is 1.85 filter unit (table 4), in the case of male adults in the sitting position, comfortably clothed, after twenty minutes of rest. Since the volume pulse as recorded photoelectrically and mechanically in the two groups of data have been obtained from analogous portions of the fingers, one may calculate that one filter unit is, therefore, approximately equivalent ot 0.033 cc. blood. Absolute accuracy cannot be claimed for this value but it serves for comparative purposes. It is important to note that the volume pulse as recorded from the entire finger by enclosing it in

a mechanical plethysmograph cannot be considered as equivalent to that recorded photoelectrically from the finger pad, after making due allowances for differences in volume of tissue involved, since the amplitude of the volume pulse is maximal in the pad of the finger tip and is very much less on the dorsum of the finger.

Sources of error. 1. *Movements.* The most important source of error and the one most difficult to control is movement of the skin with respect to the plethysmograph. Periodic movements due to the respirations and due to the ballistic effects of the heart beat on the entire body are largely eliminated from the arms and legs by flexion. These effects are so large in the case of the trunk that it has not been possible to apply the plethysmograph here. They are largely eliminated from the head in most subjects by mounting the plethysmograph on a head strap. Movements of the underlying muscles shift the position of the skin with respect to the plethysmograph. These effects may vary from fine tremors in the recorded pulse wave to gross distortion of the plethysmogram. Fortunately, the effects are quite characteristic and readily recognized so that data so distorted may be eliminated from consideration.

2. *The presence of a large artery beneath the plethysmograph* results in the recording of the pulse in this vessel. Neither the amplitude nor the form of the recorded wave is then a criterion of the arterial circulation in the skin. The plethysmogram will then fail to distinguish between large artery and skin artery reactions.

3. *Character of contact of the plethysmograph with the skin.* If the plethysmograph rests too firmly against the skin, progressive stasis in the skin vessels results showing itself in a corresponding quite uniform drift in the record. Fairly heavy pressure is required to produce noticeable stasis. Light pressure does not seem to interfere with the recording of vascular reactions nor does it produce recordable stasis. If the plethysmograph comes out of contact with the skin, light reflected from the skin reaches the photocell. The effect mimics vasoconstriction in the skin and exaggerates the influence of vasoconstriction occurring in deeper tissues, causing the skin to drop away from the plethysmograph. The amplitude of the recorded volume pulse is also changed considerably. It is, therefore, preferable to use the plethysmograph over areas in which the bones lie near the surface. Considerable experience with the technique provides confidence in one's ability to repeatedly duplicate recording conditions by repeated application of the plethysmograph to the same skin area (table 1). The range of variation in the amplitude of the volume pulse in the different trials is no greater than what may be observed during continuous recording without disturbance in the position of the plethysmograph. The values for the finger tip are lower than those reported in table 4. The differences are probably due to difference in room climate.

The constrictor effect of cold is also shown illustrating the ability of the plethysmograph to reveal such changes.

4. *The size of the vascular area under observation.* The size of the vascular area vertical to the plane of the incident light involved in the plethysmogram may include not only the areas directly beneath the photocell and the light source but possibly also a variable amount of adjoining tissue. The extent of the latter is not necessarily determinable by the extent of the visibly luminous area since plethysmography is possible also with invisible infra-red wave lengths which show greater penetration. *However*, the most intense illumination is of tissues directly beneath the light and it, therefore, follows that the effects of variations in the blood content

TABLE 1

Showing the degree of uniformity in the recorded amplitude of the volume pulse on repeated applications of the plethysmograph to the same skin area

Room temperature: 74°F. Relative humidity: 34

SKIN AREA	STRING EXCURSION TO FILTER	STRING EXCURSION TO PULSE	PULSE AMPLITUDE CALCULATED IN FILTER EQUIVALENTS
	mm.	*mm.*	
Side of nose	10.0	9.0	1.0
	9.5	9.0	0.95
	11.0	10.5	0.95
	10.5	8.0	0.76
	9.0	9.0	1.0
Finger tip	10.5	14.0	1.33
	9.0	10.5	1.17
	8.0	12.0	1.50
	8.0	11.0	1.38
Same finger tip after immersion of hand in cold water for 2 minutes	10.0	3.0	0.30
3 minutes later	13.0	7.0	0.54

will be maximal here, progressively decreasing as the light intensity diminishes, the latter decreasing in geometrical progression as the thickness of the absorbing medium increases in arithmetical progression. It, therefore, seems improbable that significant error will appear in the plethysmogram due to illumination of vascular areas beyond those immediately beneath the light and photocell. In order for light to reach the photocell from these more distant areas, it must again pass back through the tissues. The amount of light so reaching the photocell is probably very small and variations in it with the distant vasculature must indeed be small compared with those resulting from vascular reactions beneath the photocell and light. These statements are supported by the following observations on the skin of the forehead: when light from a source identical to that of

the plethysmograph was directed on the skin, it could be detected by the plethysmograph at a horizontal distance of 40 mm. providing the amplifier was operated at maximum sensitivity and providing the skin beneath the plethysmograph was not illuminated except by the distance light source. However, if the plethysmograph was operated at its usual sensitivity and with the skin beneath brilliantly transilluminated in the usual manner, the distant light could not be detected unless moved to within 10 mm. of the plethysmograph. Even so, the resulting deflection was less than that due to the pulse. *Its presence at that distance did not affect the amplitude of the recorded pulse.* It, therefore, seems fairly safe to assume that the areas significantly involved in the plethysmograms are only the two areas beneath the light and the photocell.

5. *The depth of penetration of the light.* The problem of the depth of tissue involved in the plethysmogram is a more stubborn one and one which is important to accepting the records as plethysmograms of the skin circulation only. The results of Hardy and Muschenheim (3) on the transmission of light by skin suggest that the distance penetrated is small and that the plethysmograph does not record the circulation in the deeper tissues. The possibility of the latter directly affecting the plethysmogram is obviously eliminated in the case of skin areas over bone. Since the plethysmograph does not record total light intensity but simply fractional variations in the light reaching it (amplification and, therefore, sensitivity being altered with variation in absolute light intensity), it seems probable that essentially the same depth of tissue is involved in various skin areas, provided plethysmograph sensitivity is adjusted to light absorption. Thus, if absorption is greater in one area than another, increased sensitivity of the plethysmograph would compensate for the decreased intensity of the light reaching it, tending to thus maintain the effective depth constant. Conversely, increased intensity of illumination with corresponding greater penetration would increase the effective depth if the plethysmograph's sensitivity were not proportionally decreased. That the effect is to maintain the effective depth constant is indicated in the results described below giving the same amplitude of the volume pulse (in filter units) when employing two light sources differing greatly in intensity. The fact that the amplitude of the volume pulse (calculated in filter units) is within the normal range for finger pads of normal subjects regardless of whether the hands are calloused or whether heavily pigmented as in the case of negroes is further evidence that the effective depth involved in the plethysmogram is essentially the same. The argument would fail quantitatively, however, when variation in absorption occurred without corresponding compensatory changes in plethysmograph sensitivity. That the error cannot be large is indicated by the agreement between the mechanical and photoelectric plethysmograms simultaneously recorded.

6. *Variations in intensity and spectrum of the illumination.* The spectral distribution of energy and the intensity of the light will vary slightly in the case of fresh incandescent bulbs operated similarly and slightly below their rated voltage. Small variations in these factors are relatively unimportant to the plethysmograph since one is not concerned with the absolute intensity of the light reaching the photocell providing it is sufficient to operate the plethysmograph. The amount of light absorbed in the observed area due to its blood content and due to variations in the latter will be some fraction (undetermined) of the total light penetrating the tissues and will bear some approximately constant relation to that absorbed by the filter in the plethysmograph. It is for this reason that fair success was experienced in attempting to arrive at the blood equivalent of the filter in the case of the finger plethysmograph (1). These statements are further supported by the following experiments on the finger tips: the pulse amplitude was calculated in arbitrary filter equivalents from two successive records in which the same filter was used but in which were employed two different bulbs (one an ophthalmoscope bulb, the other a pencil flash-light bulb) obviously differing greatly in the intensity of the emitted light. The filter equivalent of the pulse amplitude was the same in each record. However, this is no longer true if the spectrum of the light changes considerably as from the "white" light of the filament operated near its rated voltage to the "yellow" light resulting from falling battery voltage. It is, therefore, preferable to operate the bulb on a storage battery and to replace the bulbs quite frequently.

Experimental illustration of the validity of photoelectric plethysmograms of the skin circulation. Simultaneous plethysmograms, photoelectrically and mechanically recorded of the fingers, are exhibited in figure 3. The mechanical plethysmogram was obtained from one entire finger, the photoelectric plethysmogram from the pad of the tip of another finger. The figure illustrates the spontaneous activity of the finger vessels. The complete agreement between the two plethysmograms is obvious. Similarly perfect agreement was exhibited in the recorded responses to painful and psychic stimuli, cold, amyl nitrite, voluntary apnea and the Valsalva experiment. Such data did not permit doubt of the ability of the photoelectric plethysmograph to follow and faithfully record the vascular changes in the skin in procedures such as these giving rise to rather short-lived vascular reactions.

It seemed desirable, however, to explore the validity of more prolonged records in which the effects of reduction of oxy-hemoglobin in the skin vessels on the plethysmogram may be estimated. Matthes (4), Kramer (5), and also Gross (6) have followed photoelectrically the reduction of oxy-hemoglobin in the blood in vitro and in vivo. It is obvious from their experiments that since reduced hemoglobin has a greater opacity than

oxy-hemoglobin, increasing amounts of reduced hemoglobin in the skin vessels may mimic dilatation. It is thus possible that this source of error may at times invalidate the photoelectric plethysmogram.

Evaluation of the magnitude of the error was attempted through the performance of two types of experiment in which simultaneous photoelectric and mechanical plethysmograms of the fingers were recorded:

1, stasis prolonged to obvious cyanosis;

2, rebreathing room air until signs of marked anoxia, cyanosis, dizziness, respiratory stimulation, etc.

In the stasis experiments, the two plethysmograms tended to separate from each other. A possibly important factor contributing to this separation is the venous drainage of blood out of the finger into the hand

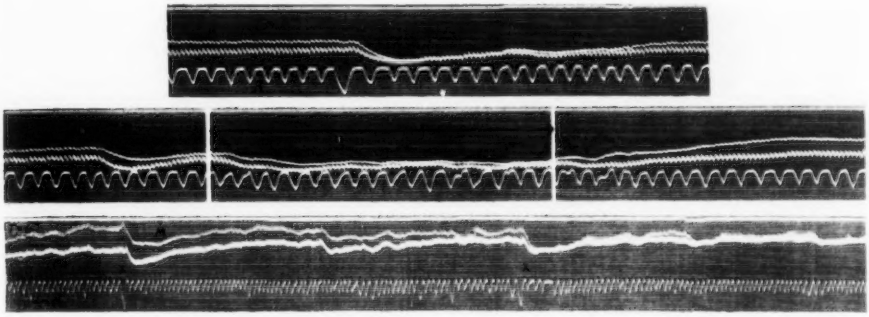

Fig. 3. Simultaneous photoelectric (*PH*) plethysmogram of pad of one finger and mechanical (*M*) plethysmogram of neighbor finger.

Upper record: constriction following deep inspiration at *X*.

Lower record: same, and in addition "spontaneous waves."

Middle record: immersion of opposite hand in ice-water between signals. Anticipatory constriction.

and arm veins which apparently form a more capacious reservoir (7) than those in the finger. This effect may be less prominent in the smaller veins of the finger tip. The evidence for the influence of accumulating reduced hemoglobin on the plethysmogram is therefore not as conclusive as one would have expected.

The rebreathing experiments presented evidence of the effects of reduction of hemoglobin only when the skin cyanosis became quite marked. Even so the disagreement between the mechanical and photoelectric plethysmograms was relatively slight. Although these experiments suggest the advisability of limiting the application of the photoelectric plethysmograph to those instances in which the ratio of reduced to oxygenated hemoglobin in the skin vessels is not greatly altered, they do not diminish confidence in the validity of the photoelectric plethysmogram in

the study of vascular reactions of the skin where this requirement is satisfied.

The volume pulse of the skin as an indicator of the state of the skin circulation (at rest). *Wave form*: As a rule, the volume pulse recorded from various skin areas, with the exception of the digits, shows a well-rounded wave in which the dicrotic wave is only slightly apparent and is placed high (usually on top) on the main wave. In the case of the digits, the wave has a triangular form with a well pronounced dicrotic wave on the catacrotic limb. In fact, the volume pulse is here indistinguishable in form from the peripheral arterial pulse, suggesting that the wave form depends upon the accidental inclusion or omission of sufficiently large arteries in the plethysmographic field to impress the arterial pulse wave form on the recorded volume pulse. Constriction in the finger tip changes the wave to a rounded form so that it resembles that in other areas. This type of wave may be due, therefore, either to absence of larger arteries or to constriction in the area observed. It is conceivable that the wave form may bear on the conclusions drawn below concerning the relative richness of the blood supply in the various skin areas studied.

Amplitude of the volume pulse as a measure of the blood supply of the skin. The amplitude of the volume pulse in a skin area will depend on the relation between arterial inflow and venous outflow in that area. If circulatory dynamics are normal in the observed area, it is probable that capillary and venous pulsation will not be detectable. However, the possibility of such a contribution to the volume pulse amplitude cannot be eliminated from consideration. This has not been studied at present with the photoelectric technique. Nevertheless, the amplitude of the volume pulse will be most dependent on the pulsatile excursions in arterial flow into the area and so will be a measure of the arterial supply. This is illustrated in table 1 in the decreased flow in the finger resulting from immersion in cold water. The argument finds further experimental support in data obtained under conditions in which other indirect evidence supports the photoelectric criteria of decreased arterial supply in the observed skin areas. Thus, in table 2, the known decrease in the arterial supply of the fingers in a case of Raynaud's disease is well indicated by the photoelectric criterion. The demonstration is given increased emphasis by the striking contrast in the amplitudes of the volume pulses of the finger tips recorded from this patient and from a normal subject just a few minutes previously. The possibility that a left cervical sympathectomy resulted in a slight dilatation in the left side of the face is also suggested by the data in table 2.

One might expect that a generalized erythema due to sunshine would offer a further convenient test of the argument. Table 3 presents such data. Although the amplitudes of the volume pulses exceed the summer averages for the corresponding skin areas, they are not beyond the upper

limits of the normal range for summer. Since the observations were made on a warm day, one is uncertain whether the increased volume pulses are due to the erythema or to the warm weather. The increases in the volume pulses do not appear proportional to the erythema suggesting that the skin arteries participate less in the reaction than do the capillaries and venules.

TABLE 2

Influence of Raynaud's disease and of left cervical sympathectomy on the photoelectrically recorded volume pulse of several skin areas (summer values)

Compare with table 4

SKIN AREA	PULSE AMPLITUDE (FILTER UNITS)	SKIN AREA	PULSE AMPLITUDE (FILTER UNITS)
Finger pad (normal subject)..	5.2–6.5	Forearm	Indeterminate
Finger pad—2nd (Raynaud's)..	0.23	Forehead (right)	0.60
Finger pad—3rd	Indeterminate	Forehead (left)	1.0
Finger pad—4th	0.58–0.8	Nose (right)	0.70
Finger pad—5th	0.10	Nose (left)	1.0
Thenar eminence	0.58–0.75	Ear lobe (right)	0.33
Hypothenar eminence	0.36–0.55	Ear lobe (left)	0.34
Dorsum hand	0.41		

TABLE 3

Amplitude of volume pulse in several skin areas in a case of erythema due to sunshine

Normal subject. Temperature: 92°F. Relative humidity: 43. Compare with table 4.

SKIN AREA	PULSE AMPLITUDE (FILTER UNITS)	SKIN AREA	PULSE AMPLITUDE (FILTER UNITS)
Forehead	2.0	Finger pad	4.6–5.2
Nose	1.68	Dorsum finger	0.75
Ear lobe	3.0	Dorsum hand	0.6
Ear (upper part)	1.0–1.4	Forearm	0.24
Cheek	1.4–1.8	Toe pad	2.5–2.8

The argument that the amplitude of the volume pulse is a criterion of the blood supply of the skin has been applied to a comparison of the richness of the blood supply of various skin areas (table 4). The data have been obtained on healthy male adults in the sitting position, comfortably clothed, after twenty minutes of rest. The values on the arms, hands and fingers were obtained with these areas at heart level; those for the leg and

foot with the leg comfortably raised to a level slightly below heart level. This is an important provision as the volume pulse is significantly influenced by the vertical distance of the part above or below the heart (2),

TABLE 4

Amplitude of volume pulse (in filter units) in various skin areas of healthy male adults

SKIN AREA	SEASON	SUBJECTS	OBSERVATIONS	RANGE	AVERAGE
Finger pad	W	3	5	0.96–2.4	1.76
	Sp.	3	5	1.0 –2.8	1.85
	Su.	11	17	2.1 –6.5	3.4
Forehead	W	8	17	0.43–1.63	0.94
	Sp.	7	7	0.43–1.54	1.00
	Su.	7	9	0.67–2.0	1.00
Forearm	W	4	11	0.15–0.48	0.31
	Sp.	6	7	0.0 –0.34	0.17
	Su.	6	8	0.0 –0.59	0.30
Ear lobe	Sp.	6	6	0.53–1.43	0.97
	Su.	6	9	0.75–3.3	1.80
Toe pad	Sp.	5	6	0.30–1.2	0.63
	Su.	5	8	0.28–2.5	1.23
Nose (side)	Sp.	6	6	0.67–1.43	1.07
	Su.	6	8	0.70–1.75	1.17
Cheek	Sp.	7	7	0.31–1.07	0.68
	Su.	3	3	1.1 –1.3	1.20
Lip	Sp.	4	4	0.83–1.80	1.16
Thenar eminence	Sp.	5	5	0.32–1.4	0.90
Hypothenar eminence	Sp.	4	4	0.46–2.26	1.04
Palm (hand)	Sp.	3	3	0.38–1.71	0.90
Dorsum finger	Sp.	8	8	0.25–0.92	0.54
	Su.	2	2	0.50–0.90	0.70
Dorsum hand	Sp.	6	6	0.0 –0.68	0.37
	Su.	6	8	0.20–0.70	0.47
Dorsum foot	Sp.	5	5	0.0 –0.45	0.11
	Su.	3	4	0.0 –0.33	0.20
Knee	Sp.	6	6	0.0 –0.46	0.20
Tibia	Sp.	6	6	0.0 –0.21	0.04
Dorsum toe	Sp.	5	7	0.0 –0.38	0.15

Spring (Sp) and winter (W) temperatures in room 75–80°F; relative humidity 30-
Summer (Su) temperatures in room 82–93°F; relative humidity 38–47.

a fact which our own experience confirms. The data have been grouped into three divisions according to the season of the year. However, the room climate was essentially the same during the winter and spring observations so that the separation of these is probably not necessary as

indicated by the essential identity of the winter and spring averages for the finger pad, forehead and forearm areas. The data indicate that a rich arterial blood supply is limited to the skin of the palmar surface of the hand and fingers, the plantar surface of the toes, the forehead, face and ear. The dorsal aspects of the hands and feet and the plantar surface of the feet have a much poorer arterial supply. The arterial supply to the skin of the arm and leg is so poor that the volume pulse in these areas is not recorded satisfactorily. In many instances, the amplitude of the volume pulse is indeterminate.

One might expect that vasomotor reactions would be more pronounced in the skin areas with the richer arterial supply. Grant and Pearson (7) have recently shown striking differences in the vascular reactions of the forearm and of the hand. Others (8) have suggested greater vascular reactivity in the hand than in the foot. However, our own experience suggests that such differences in vascular reactivity are not necessarily dependent upon the richness of the arterial supply. Thus, in table 4, we observe a surprising seasonal constancy in the volume pulse of the forehead, nose, forearm and dorsum of the hand and foot in contrast to the dilatation in warm weather in the finger pad, toe pad and ear. Similarly observations on the vascular responses of the forehead, ear and finger to the cold pressor test (9) indicate the lability of the arteries of the finger in contrast to the absence of significant reactions in the forehead and ear.

These data support the suggestion of Grant and Pearson (7) that the dissipation of heat from the hand and forearm is greatly affected by digital blood flow and that the venous flow in the skin of the hand and forearm is derived importantly from the digits and the muscles below the skin. They are in further agreement with the constancy of forehead skin temperature, a fact well-known to clinicians and recently commented on by DuBois (10).

SUMMARY

1. Advantage may be taken of the fact that the absorption of light by a transilluminated tissue varies with its blood content, to detect vascular changes with the photoelectric cell. The application of this principle of photoelectric plethysmography to the study of the skin circulation is described. The sources of error involved in the quantitation of the skin plethysmogram are discussed. They bear on the problem of the blood equivalent and on the qualitative accuracy of the plethysmogram. They are:

a. Movement of the skin with respect to the plethysmograph.

b. Influence of large artery in the immediate neighborhood of the area being observed.

c. Character of contact of the plethysmograph with the skin.

d. Size and depth of the vascular area involved in the plethysmogram.

e. Variations in the intensity and spectrum of the illumination.

f. Influence of reduced hemoglobin—oxygenated hemoglobin ratios on the skin opacity.

2. The essential validity of the photoelectric plethysmogram is demonstrated in simultaneous plethysmograms, photoelectrically and mechanically recorded, of the fingers, in instances where the vascular responses to some common procedures were followed.

3. The argument is advanced and supported by suitable data that under resting conditions, with normal circulatory dynamics, the volume pulse of the skin area is a measure of the richness of the arterial blood supply of that area.

The skin areas thus studied arrange themselves in descending order of the richness of their arterial supply as follows: finger pad, ear lobe, toe pad, palm of hand, skin of forehead and face, dorsum of finger, of hand and of foot, forearm, knee and tibia. Other areas could not be estimated.

4. The effect of climate on the arterial blood supply of these areas is also indicated.

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THE ACTION OF ISOTONIC, SALT-FREE SOLUTIONS ON CONDUCTION IN MEDULLATED NERVE FIBERS

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Isotonic glucose solution applied to the sciatic nerve of the frog at 12°C. does not block conduction in its fibers even after 17 hours (Netter, 1926). Applied directly to a Ranvier's node of an isolated single fiber it blocks almost instantaneously (Kato et al., 1936; 1938). Applied to a slender spinal root of the bullfrog—the IVth or the Vth or even the VIth—blocking, we find, may become complete within the course of a few seconds. The Japanese investigators have succeeded in showing through operation on isolated nerve fibers, that the internodal regions of the fiber are protected by the medullary sheath from electrical currents, narcotics and salt-free solutions, and believe they have proven that currents eddying outside of the medullary sheath are not essential to propagation of the nerve impulse. The possibility that nodes are particularly susceptible to injury, that injury accounts for the results they have obtained, is reasonably answered by their experiments on single nerve fibers of the basihyoidal membrane and on nerve trunks, in which, by means of electrical stimulation, low threshold points could be located which have spacings simulating those of nodes.

In 1934 it was shown (Erlanger and Blair) through observations on the phalangeal preparation that "nodes are less well insulated than internodes, both electrically and chemically." Having found a structure, the spinal root, that merely needs to be lifted from its surroundings, that responds with surprising promptness[1] and with a fair degree of reversibility to treatment with a salt-free, inert, isotonic solution, the present investigation was undertaken in an effort to ascertain how such a solution acts in modifying nerve conduction, whether fiber size is a factor in its action, et cetera. At the outset it may be stated that our present observations confirm the Japanese investigators in showing that salt-free solutions exert their effects via nodes of Ranvier.

METHODS. The roots of the bullfrog (*Rana catesbiana*) were excised

[1] Bishop (1932) has noted that KCl acts much more quickly on nerve roots than on nerve trunks.

together with trunks and branches to which they are tributary, and stored in Ringer's solution (formula given by Bayliss, 1920) at 5°C. until used. Records of the action potential in the root were made with the electron oscillograph after amplification.[2] When the response of practically all of the fibers of the root was desired the attached trunk was stimulated maximally; when it was desired to observe the responses of a few fibers with a wide range of conduction rates a branch was sought by trial, maximum stimulation of which gave the desired picture in the root. When, under the latter circumstances, there happened to be a large fiber with an outstanding excitability, it became possible through the use of methods to be described, to follow the changes occurring in the responses of that fiber as the effects of the isotonic solution of glucose (to be designated simply "glucose solution") developed and receded. The responses of single fibers are sufficiently ample for detailed study only in slender roots; therefore the IVth and the Vth usually were used for this purpose.

The root usually was mounted vertically, or nearly so, but the rest of the preparation was carried at an acute angle around a broad support (an intermediate ground) from below upwards. Then the solutions employed either were dripped over the vertical root, excluding the immediate region of the leads (except where otherwise stated), or they were applied, through applicators, accurately to selected localities. These applicators were glass tubes with terminal orifices of suitable widths. The applicator approached the root with a slight upward inclination so that the solution, when flowing through the tube, ran back along it, and did not, when contact was made, drip along the root. Other devices for localizing or generalizing the application of the solution will be described in due course.

In most of the experiments in which the root was alternately treated with Ringer's and glucose solutions, both had the hydrogen ion concentration of the laboratory distilled water. Fenn et al. (1934) have found that the loss of potassium by nerve is not affected by moderate variation in hydrogen ion concentration, and we have been unable to detect any differences in behavior referable to the presence or absence of buffers in the Ringer's solution. The solutions may produce effects so promptly that in order to keep track of elapsed time it became necessary to photograph along with the pictures on the screen of the tube, the face of a running watch. Pictures could be taken at one second intervals; shorter intervals between happenings could not be measured.

RESULTS. *The solution acts at nodes.* After considerable experimentation it became obvious that only through controlled topical application of the solution to the root while recording the action potential of a single

[2] For more detailed descriptions of methods see Blair and Erlanger (1933) and Erlanger and Gasser (1937).

fiber could any progress be made toward ascertaining how the solution produces its effects. Resort was then had to the "applicators" and it was found, using the narrowest applicator (one with a transverse orifice 0.5 mm. wide that made contact with about 1 mm. of nerve), that in order to block propagation in a fiber within a reasonable time it was necessary to apply the solution to specific points along the length of the root. These points, it was found, are spaced roughly by 1.25 to 2.5 mm. or more. Systematic measurements have not been made. Moreover, it always was possible to find intermediate points where application of the solution had no obvious, or at most only a minimal, effect on conduction within any reasonable time.

When the locus of application at which block in a fiber develops rapidly is not too far removed from the recording electrode (not over 3 to 4 mm.

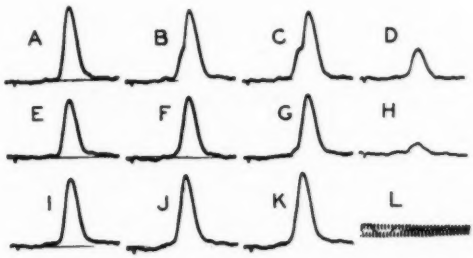

Fig. 1. Stages in the blocking of the impulse in a fiber induced by a 1 mm. application of the solution at two adjacent loci of highest susceptibility, one, *A*, *B*, *C*, *D*, 1.2 to 1.5 mm. from the lead, 0, 2, 3, and 4 seconds after contact, respectively; the other, *E*, *F*, *G*, *H*, 3.0+ mm. from the lead, 0, 3, 5 and 7 seconds after contact. *I*, *J*, *K*, made 0, 10 and 16 minutes after contact, respectively, show that application in an intermediate position does not block. The time, *L*, 10,000 cycles, applies to all records. Further details in text.

and preferably closer) the records of the spike change progressively in a characteristic manner, such as is seen in figure 1. The records of the top row were made with the applicator about 1.3 mm. from the lead electrode. Stimulation, as usual, was at the rate of 1 per second and, beginning immediately after the application of the solution, every response was recorded excepting the first which was missed because of the amplifier disturbance resulting from the completion of the contact between applicator and nerve. Within two seconds a notch appears on the ascending limb of the spike about two-fifths the way up (*B*). At 3 seconds (*C*) this notch has deepened to the extent that the record there becomes almost horizontal. At 4 seconds (*D*) the portion of the record above the notch has vanished and there is left a spike about two-fifths the height of the original one, but

of about the same temporal configuration.[3] During this metamorphosis the height of the notch above the base line does not change appreciably; certainly it does not decrease, though the peak of the spike does fall somewhat.

Then, with intervening intervals of washing with Ringer's solution, which throughout this experiment immediately (i.e., within 1 sec.) restored the spike, the applicator was applied to the root at successive points further from the lead electrode until another locus was found at which application, again, rapidly was followed by a series of pictures comparable to the first set. These are seen in the middle row of figure 1. At this new locus the break occurs after 7 seconds, but only three of the steps in the process (*F*, *G* and *H*) are reproduced. The remnant that is left (*H*) now is lower than in the first series;—it is a very small fraction of the original spike, so low that it is considerably deformed by the noise of the amplifier. The remnants in both cases remain unchanged during continued treatment of the nerve with glucose, excepting some diminution in amplitude, of which more in a moment.

At points about midway between these two loci of least resistance the solution, in this experiment, could be left in contact with the root for periods as long as 16 minutes without fragmenting the spike or altering its height appreciably. The records obtained with the applicator in such an intermediate position are seen in the bottom row of figure 1. Record *I* was made immediately after the applicator, dripping the solution, came into contact with the root, records *J* and *K* about 10 and 16 minutes later, respectively. Close examination of the last of these records reveals faint indications of a discontinuity on the rising phase of the spike. This discontinuity, however, occupies the same position as those developing in the top row; it is not located between the notches seen in the top and middle rows of spikes where one would expect it to appear if the solution were exerting its effect exactly at the point of application. It seems justifiable to conclude, therefore, that in this intermediate position the small effect the solution exerts is produced not where the applicator rests, but possibly through diffusion (see p. 346), upon the first of the two loci of least resistance located in this experiment. Action on the intermediate location over a period of 16 minutes therefore was wholly without *local* effect, when 4 to 7 seconds sufficed to block the impulse at the most favorable locations.

These loci of least resistance to the action of the salt-free solution undoubtedly are nodes of Ranvier. The only other possibility is that they are injured points. One reason for turning from the phalangeal nerve (Erlanger and Blair, 1934) to roots for this investigation was the fact that to prepare the latter it merely is necessary to lift them out of the sub-

[3] When the unblocked spike is diphasic, it becomes monophasic at this time (see fig. 2 *D*).

arachnoid space. There is no reason, therefore, for suspecting local injury as the cause of these results. Moreover, it is inconceivable that local injuries would be spaced as are these loci of least resistance. And, finally, local injury would be apt to involve all of the fibers locally; but it has been possible to find in a preparation a locus where the largest axon spike could be blocked in 14 seconds (and not in 5 min. + off of it), whereas continuing the action at the same locus for 78 seconds + was without effect on another, a more slowly conducted, spike (see table 1, columns 12 and 13).

TABLE 1

1	2	3	4	5	6	7	8	9	10	11	12	13
			BROAD APPLICATION, SOLUTION NOT FLOWING								NARROW APPLICATION, SOLUTION FLOWING	
			1st locus				2nd locus		3rd locus		1st locus	2nd locus
			1st trial		2nd trial							
FIBER	THRESHOLDS	COND. RATES	Blocking time	Recovery time*	Blocking time	Recovery time†	Blocking time	Recovery time‡	Blocking time	Recovery time§	Blocking time¶	Blocking time¶
		M/sec.	*sec.*	*sec.*	*sec.*	*sec.*	*sec.*	*sec.*	*sec.*	*sec.*	*sec*	*sec.*
A	1	23.0	65	0	51	0	100	0	120	0	14	13
B	2.88	9.37	107	1	107	1	65	45	40	Others not	78+	30
C	3.79	6.82	42	16	49	2	31	74	31	noted	Absent	6
D	5.70	4.33	45	7	23	1	24	77	20		Absent	Absent
E	6.80	3.75	65	7	100	1	81	74	90		4	3

* One drop of Ringer's applied 15 sec. after dripping with glucose was stopped.

† One drop of Ringer's applied 44 sec. after dripping with glucose was stopped.

‡ One drop of Ringer's applied immediately after dripping with glucose was stopped.

§ Recovery time of fiber *A* alone noted.

¶ Over node of fiber *A*; application off if node did not block in 5 minutes.

The experiments signify, therefore, that the solution exerts its effects entirely via the nodes; that the medullary sheath, despite the presence of the incisures of Schmidt-Lantermann, protects the axon against a salt-free solution at least 170 times more effectively than the axon is protected at nodes.

The potential which records when a fiber is blocked by the solution at a node not too distant from the recording electrode is the "extrinsic," or electrotonic extension of, potential from the active locus where blocked. The phenomenon has been seen by Erlanger and Blair in fibers blocked at nodes by anodal polarization and by solutions poor in calcium or potassium

(1934). It has been seen also by Hodgkin beyond blocks produced by compression or by cold (1937).

It has been stated that prior to the onset of block the height of the deepening notch on the ascending limb of the spike remains constant. This signifies that during this period the height of the extrinsic potential remains constant. With the onset of block, however, the extrinsic potential may immediately begin to diminish. This decline in height, rapid at first, eventually approaches an asymptote. Figure 2 presents a typical result. The top row shows that during the 41 seconds required to block the impulse there was no diminution in the height of the extrinsic potential, as indicated by the height of the notches in *A* to *D*. In the middle row the higher spike in each case is the extrinsic spike of *D*. Superimposed on it in *E*, *F*, *G* and *H* are the extrinsic spikes recorded during the continued local

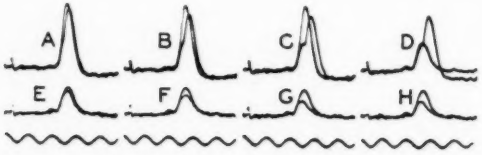

Fig. 2. Stages in the blocking of the impulse in a nerve fiber by continuous application of the solution over a node of Ranvier slightly over 1 mm. from the lead.

Top row: The normal axon spike superimposed on the spikes recorded 12 sec., 27 sec., 37 sec. and 41 sec. after applying the solution in *A*, *B*, *C* and *D*, respectively.

Middle row: The extrinsic potential of *D* (recorded immediately after block developed) superimposed on the extrinsic potential recorded later, 21 sec., 84 sec., 4 min. and 10 min. in *E*, *F*, *G* and *H*, respectively. The shock artifacts, the initial low peaks, also are superimposed. Time, 1000 cycles.

exposure of the nerve to the solution after the block developed, respectively, 21 seconds, 84 seconds, 4 minutes and 10 minutes.

It has been seen that prior to the onset of block the height of the action potential diminishes. But since the height of the extrinsic potential remains constant during that period, while the upper element of the spike moves later with respect to the lower, there is the possibility that the diminution in height is merely apparent and due to the change in the relative positions assumed by the two elements. As a matter of fact, measurement shows that the decline in height closely parallels the part of the falling phase of the extrinsic potential with which the successive crests of the upper elements are summing. The significance of some of these observations will be considered later.

Behavior under repeated treatments. There are many vagaries in the behavior of a fiber under treatment with the solutions which must be

considered in evaluating results. For instance at a time when everything seems to be in a steady state one fiber of several discrete ones under observation may stop conducting and, having stopped, may, apparently spontaneously, begin to transmit impulses again. Instances of such behavior will be cited in due course. Presumably this behavior is related in some manner with the matter of salt balances, a problem that does not come within the scope of this investigation. A comparable condition can be produced by subjecting the root to repeated or to prolonged treatments with the glucose solution in the manner indicated in the following experiment.

A slender root was arranged so that a considerable part of its length could be dripped with glucose or Ringer's solutions and the behavior of the

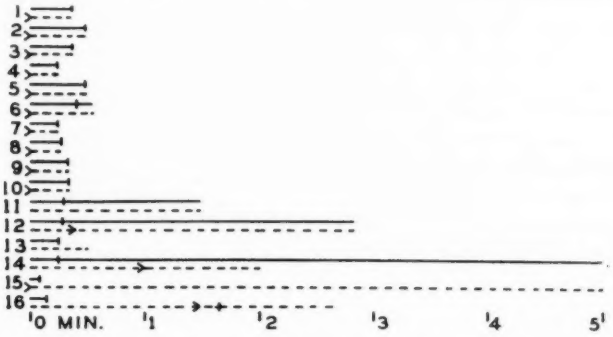

Fig. 3. Diagram showing blocking and recovery times in a fiber alternately treated with glucose (continuous lines) and Ringer's (dotted lines) solutions. The successive pairs of treatments are numbered on the left; the duration of the several treatments, in minutes, is indicated below. A cross bar on the horizontal lines indicates the onset of block, an arrow head the return of conductivity.

action potential of the most irritable fiber was observed through the course of treatment to be described. The root, which as usual had been stored in Ringer's solution, was alternately treated with glucose (solid horizontal lines in fig. 3) and Ringer's (dotted lines) solutions for the periods of time indicated roughly by the lengths of the horizontal lines. Stimulation, as usual, was at the rate of 1 per second. The moment of entrance into block is indicated by the cross bars on the horizontals, of recovery from block by the arrowheads. The graph shows that through 10 alternate treatments, first with glucose just long enough to block the fiber, and then immediately with Ringer's for about the same period of time, there was perhaps a slight general tendency for the blocking time in glucose to shorten; the fiber, however, continued to recover promptly (within the

period needed for the amplifier to settle down) with each return to Ringer's.[4] Then, beginning with the eleventh pair of treatments, the exposure to glucose was prolonged beyond the blocking time. Now, with the successive trials, the time required to block began to shorten more definitely, despite a corresponding increase in the duration of the intervening treatments with Ringer's solution. Moreover, now (see the 12th, 14th and 16th treatments) the fiber no longer recovered promptly when it was washed with Ringer's and this delay increased through the 16th treatment, when 90 seconds elapsed before recovery occurred. But 10 seconds later the fiber again ceased conducting despite the continued exhibition of Ringer's solution. It may be added here in passing, that a fiber that has failed to conduct under these circumstances may begin to conduct again when treated with isotonic sodium chloride solution.

Comparative observations on fibers of a preparation. If there is an inherent difference in the responses of medullated fibers to the glucose solution dependent upon their position in the conduction rate spectrum, how could this be determined? Since the action of the solution is through nodes and since there is the possibility that the nodes of a fiber are not all equally susceptible to the solution, it is clear that to make comparison valid a large number and the same number of nodes of each of the fibers should be exposed and to exactly the same treatment. Some of these conditions cannot in the nature of things be met, while others can be met only through the observance of certain precautions.

1. The length available for treatment is not over 5 mm. in smaller roots nor more than 9 to 10 mm. in larger roots. How many nodes would these distances include? It is usually stated (Gerard, 1931, p. 70) that in the frog 2 mm., in round numbers, is the maximum internodal distance. But Boycott (1904) found among 1916 segments in large frogs (temporaria?) 70 that exceeded 2.0 mm. in length, the longest measuring 2.85 mm. Our own few indirect measurements indicate that many of the segments in root fibers of the bullfrog exceed even this length. And the Japanese investigators (1936, 1938) picture in one and the same large fiber from the sciatic of the toad, internodes ranging in length between 3.69 and 1.08 mm. In other of their illustrations lengths of over 3 mm. seem almost to be the rule. On the basis of their data, it is obvious that to be able to operate upon 3 nodes of a large fiber, the length of root available must exceed 9 mm., and three are too few to care for the possibility of variation in the structure and position of nodes.

2. If, moreover, segment length varies with fiber diameter, and the literature indicates that it does, small fibers will have more nodes exposed to the treatment than large, and if nodes vary in their susceptibility, the

[4] It will be shown, however, that fibers differ considerably in their ability to recover.

former will to some degree be at a disadvantage. The best data available on the relation between fiber diameter and segment length are those that have been analyzed by Hatai (1910). However, the average values for the different sizes of fibers have such a wide scatter that it seems precarious to use Hatai's data as a basis either for discussion or for experiment. Depending upon which of his sets of fibers is used one can take as the proportionality constant, upon the arbitrary assumption that the relation is one of direct proportionality, either 0.33 or 3.6. Upon this basis the statistical error due to differences in the number of nodes in the fibers treated might be either insignificant or very considerable.

3. Whatever the length treated, diffusion conditions are such that there will be a concentration gradient, falling toward the middle of the treated stretch, which will be steeper the shorter the length and the thicker the root.

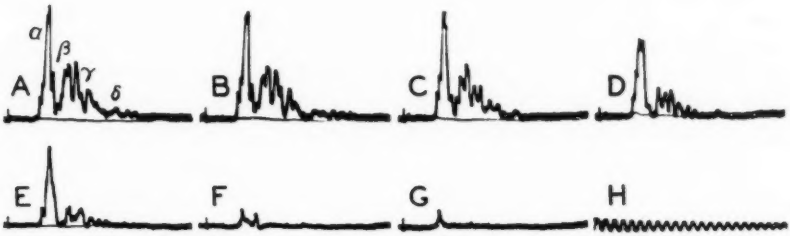

Fig. 4. Seven stages in the action of glucose solution on conduction in more or less discrete fibers of a IXth sensory root; 9.5 mm. treated. Time, 1000 cycles, applies to all records. The initial low peak in each record is the shock artifact. Further description in text.

4. And, finally, should any particular locus along the length treated be favored in any other way, for instance by local lingering of a drop of the solution coursing over the root, the fibers having nodes in that locus would appear to be unduly susceptible.

Without exception, therefore, every source of error will have the effect of giving the impression that small fibers are more susceptible to the treatment than large.

Just how the constituent fibers block when the comparative observations are made under optimal conditions is illustrated by the following experiment. The preparation was a large sensory root, the IXth, with *many* fibers responding; but the distance to the stimulated branch was so long (160 mm.) that the pictures (fig. 4) nonetheless yield considerable information regarding the behavior of individual fibers. The solution was applied *uniformly* to 9.5 mm. of root length by immersion in a dependent position in a pool on a glass slide. Stimulation was maximal.

In the normal record (*A*) one can distinguish for purposes of description 4 groups of spikes; they may be *alpha*, *beta*, *gamma* and *delta*, and therefore are so labelled.

From the many records made 6 have been selected to illustrate how the picture changes as the glucose solution takes effect. At 127 seconds (*B*) *delta* has separated into 5 peaks due to latening, particularly of the slower spikes. *Alpha*, *beta* and *gamma* still seem to be intact but their outlines have changed slightly due to some lagging of their constituent spikes. After 190 seconds (*C*) only one of *delta's* spikes is left, *gamma* is disintegrating and has lost some of its elements, *beta* also has lost some, though not so many, and possibly *alpha*, too. After 229 seconds (*D*) one of the *delta* spikes still is left, *gamma* has lost most of its area, and *beta*, proportionally almost as much, while *alpha* is broadening and possibly has lost some of its area, also. At 261 seconds (*E*) the persistent *delta* spike has disappeared and all of the earlier conglomerations have lost in area, *alpha* the least. After 355 seconds have elapsed (*F*) there are left only two of *alpha's* spikes, at 362 seconds only one, and this one obviously is the fastest in the normal root. Thus, though it is difficult to follow accurately every step in the process of disintegration, it nevertheless is obvious that in general the slower spikes block first.

It also can be seen in these records that the lag that develops before conduction stops is greater in small than in large fibers. The spike of the slowest fiber lags about 1.45 msec., that of the fastest about 0.6 msec., but the experiment was discontinued before this, the most persistent, fiber had ceased to conduct. How much more the spike would have lagged it is impossible to say. Under the circumstances, a fair estimate would make the greatest lag about twice as large as the least lag that developed in this preparation. Now, it has been shown *a*, that all of the lag produced by treatment with the glucose solution develops at nodes, and *b*, that block results when the lag attains, or exceeds slightly, the time to maximum of the extrinsic spike. The time to maximum of the extrinsic potential of the fastest fiber is of the order of magnitude of the time to maximum of the spike itself, and can be assigned a value of 0.3 msec. But the time to maximum of slower spikes is longer; the increase, though, does not become considerable until conduction rates fall below 7 M/sec. (Blair and Erlanger, 1933). If we take the time to maximum as the same for all fibers then the lag becomes proportional to the number of nodes. If, then, the average of segment length of *alpha* fibers be put down as 3 mm., that of the smallest fibers in the present preparation becomes 1.5 mm. But, for reasons given, this range is, if anything, too wide.

The conclusion that this rather indefinite evidence seems to warrant (it is supported, however, by other experiments) is that when as much as 9.5 mm. of root length is exposed to treatment the number of nodes treated

will range between 3 in the largest fibers and 4 to 5 in the smallest, approximately. Therefore if the nodes of a fiber vary in their susceptibility to the treatment the small fibers stand a slightly better chance, statistically, of succumbing first. The relative susceptibilities of fibers of different sizes displayed in the present experiments may not, therefore, be real.

The next experiment is selected for consideration primarily because of the light it seems to throw on the mechanism of salt action, but also because of certain of the vagaries of its responses. The solution was put on the root through an applicator 3.0 mm. wide. The observations were made on the fibers of a sensory root that had been stored for 6 days in the refrigerator. With maximum stimulation there were 5 readily distinguishable axons; they are labelled *A* to *E* in figure 5 and in table 1. Their relative thresholds ranged from 1 to 6.8 and their conduction rates from 23.0 to 3.75 M/sec. Presumably they all were medullated fibers. In order to obtain a wide range of blocking times the freshened solution in the applica-

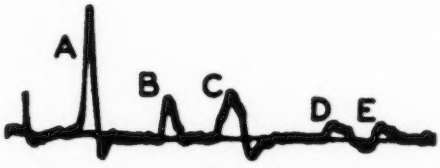

Fig. 5. Five axon spikes (*A* to *E*), with conduction rates ranging between 23.0 and 3.75 M/sec. Their behavior when the root is treated with glucose solution is described in the text and in table 1.

tor was permitted to stagnate when applied to the root. Nine millimeters of root length were available for treatment. In the part of the experiment to be considered now the solution was applied to the root four successive times with ample intervening periods of treatment of the nerve with Ringer's solution. The first two treatments involved, as nearly as was possible with a reapplication, the same middle stretch of root; the third was applied closer to the lead and the fourth closer to the ganglionic end of the root. The last two applications overlapped slightly the previously treated middle region.

The results of these four trials are collected in table 1, columns 4 to 11. It is seen there that two of the three more slowly conducting fibers, *C* and *D*, were the first to block, whereas the slowest fiber, *E*, was as resistant as the two fast fibers, *A* and *B*.

In each of the trials, after all the fibers had blocked, the applicator was removed and then, after different intervals up to 44 seconds (see table), the root was doused with just one drop of Ringer's solution. A number of points of interest emerge of which two are mentioned. 1. In every

trial the fastest conducting fiber, *A*, recovered immediately upon withdrawing the glucose solution before letting fall the rinsing drop of Ringer's solution. This happened despite the fact that fiber *A* was by no means always the last fiber to be blocked by the salt-free solution. One might be inclined to conclude from this behavior merely that the most vulnerable node of *A* was close to the edge of the treated region. This interpretation, however, is invalidated by the fact that in the four separate trials involving at least three different stretches of the fiber, the reaction was essentially the same. This behavior, it may be added, is very different from that of a fiber that is beginning to fail as a result of treatment with glucose solution. Under the latter circumstances, it has been seen, the longer the fiber remains blocked in glucose solution the later does it recover in Ringer's solution.

2. The other fibers that had been blocked by the glucose solution recovered only after the drop of Ringer's had coursed over the root (see table 1). And in every trial the longer the delay in applying the drop of Ringer's the shorter were the respective recovery times. Thus when the interval was 44 seconds (columns 6 and 7) all of the fibers recovered within the first second or two; and when the delay was made as brief as possible (columns 8 and 9) the recovery times for the same fibers ranged from 45 to 77 seconds. The fastest conducting of the fibers (*B*) recovering in the latter manner was the earliest to recover.

One must conclude on the basis of these observations, that, from the standpoint of resiliency, fiber *A* ranks first, *B*, second and *C*, *D* and *E*, indistinguishably, last. Though the large fibers may block as soon as the small, they recover more rapidly.

This ability to recover from the solution in the absence of a Ringer bath, or to recover sooner after a Ringer bath when there has been an interval during which the blocked fibers have been untreated, must signify that fibers contain resources for recovery within themselves. In this sense the large fibers are more self-reliant than the small. Presumably the recovery is accomplished from within by a restitution of a necessary salt atmosphere in, or in the immediate vicinity of, the node. The only other source of salts would be untreated nerve space and it seems extremely unlikely that they could migrate the necessary distances within the time available, particularly in view of the fact that in the intervals the surface of the treated portion of the root remains wetted with the glucose solution. And in any event such diffusion would affect all of the fibers alike, at least statistically. Under the conditions of these trials the blocking time bore no relation to the size of the fibers. Was it because the treated stretch was short?

When, after these treatments, the root had recovered in Ringer's as fully as it would, the effect was determined of applying the narrowest

applicator (0.5 mm.) to a hitherto untreated region closer to the lead electrode. As noted on an earlier page (the data are in table 1, columns 12 and 13) 1, fibers *C* and *D* had not recovered from the previous treatment, although *C* did later, and 2, the applicator, now with the solution flowing (in previous determinations it had been stagnant), was placed on the locus at which fiber *A* blocked speedily (in 14 sec.). Fiber *B* now in one of the trials (column 12) did not block during the period of observation (78 sec.), and in the second trial (column 13) blocked in 30 seconds; but the small fibers, *C* and *E*, blocked in 6 and 3 or 4 seconds, respectively.

It should be added that the general reduction in the blocking times recorded in columns 12 and 13, as compared with previous determinations, is attributable to the change from a stagnant to a flowing application of the solution. But the change in the order of blocking can be accounted for only by assuming that the position happens to find in fibers *C* and *E* nodes that are particularly vulnerable to the solution.

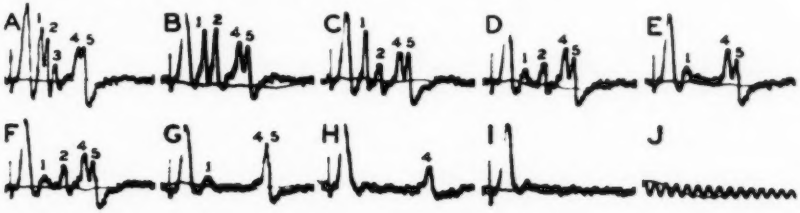

Fig. 6. Stages in the action of glucose solution on conduction in 5 discrete axons of a small VIth sensory root; 6 mm. treated. The first high wave in each record is an extrinsic potential, presumably from the multifiber motor spike, led indirectly; it is without significance. Time, 1000 cycles, applies to all records. For details, see text.

The results obtained with the pool immersion method when the roots are small, and the responding fibers few in number, do not differ materially from those obtained by the applicator method. It will suffice to give the results of one of these experiments. The preparation was an unusually small VIth sensory root removed two days previously. Six millimeters of the root could be immersed in the pool. As may be seen in figure 6, the changes developed tardily, due, partly at least, to the slow dilution of the Ringer pool by the glucose solution.

The recognizable axon spikes, 5 in number, are designated *1* to *5* in the order of their conduction rates, which ranged from 15.0 M/sec. down to 5.8 M/sec. The order of blocking in seconds, as shown by the successive records in the figure, is *3*, (record *B*) 464, 1st node of *2*, 466 (record *C*), 1st node of *1*, 533 (record *D*), 2nd node of *2*, 562 (record *E*) (in *F* the 2nd node of *2* unblocks and in *G* it blocks again), 2nd node of *1*, 792, *5*, 818 (record *H*), and *4*, 838 (record *I*).

Later, after the nerve had recovered in Ringer's solution, the order of blocking of the fibers from a 1 mm. application of flowing glucose solution was determined. The application was made to the part of the root not previously treated, close to the lead. Now the fibers blocked (and vacillated), as follows, in seconds: *3*, 192, *1*, 404, *4*, 474, *5*, 545, *1* conducting again, 454, *2*, 861, *1* finally, 1780. Here we have another illustration of the vagaries of the reactions of axons to this treatment: fiber *1* might be regarded either as sensitive or insensitive to the action of the glucose solution!

There is an obvious difference in the order of blocking in the two sets of determinations. With the broad applicator the slower fibers on the average block at least as late as the faster one. With the narrow applicator the slower fibers all are blocked at a time when some of the faster fibers still are conducting. Such experiments as this one, with only a few fibers on which to base a conclusion, indicate that broad application of the solution favors the blocking of large fibers, narrow application the blocking

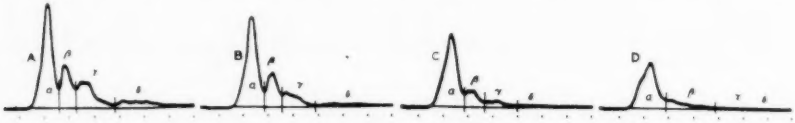

Fig. 7. Complete action potential of a small VIth sensory root. Four stages in the effect of glucose solution (3 mm. application) on the areas of the *alpha*, *beta*, *gamma* and *delta* elevations, as delimited by the verticals drawn on the records. Time, 1000 cycles. See table 2.

of small fibers. But this conclusion, as has been seen, is not borne out by experiments in which the effects are observed on large numbers of fibers.

One more instance, this one dealing with very large numbers of fibers, may be given in support of the first experiment cited. In this case the preparation was a VIth sensory root with clear *alpha*, *beta*, *gamma* and *delta* elevations, smooth in contour because of the many contributing spikes (fig. 7). Two different stretches of the root were treated with the glucose solution, first one with an applicator about 3 mm. wide, then the other, after recovery from the first treatment, with an applicator 1 mm. wide. The latter was applied to previously untreated parts of the root between the point of first application and the lead. On enlarged copies the elevations were delimited as shown in the figure and their areas measured. They decreased under treatment as shown in table 2, *delta* at the fastest rate, *alpha* at the slowest, *beta* and *gamma* at intermediate rates. The principal, if not the only, difference between the effects produced by the two applicators is in respect to the time required to produce a given percentile decrease in area in the several categories;—the narrow applicator

produces the same effects as the wide one, but very much more slowly. The result seems to signify, again, that if the internodal distances are proportional to the diameters of the fibers, the differences are not sufficiently great to be demonstrable through the use of applicators of different widths in the case of larger roots.

It may be added that in this last experiment when *delta* and *gamma* were invisible under the amplifications at which the records of figure 7 were made, there was no sign of *delta* under the highest amplification. Unquestionably all of the *delta* fibers were blocked. We have no explanation to offer for the difference in the behavior of large and small roots in this respect, other than the statistical one.

TABLE 2

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	3 MM. APPLICATOR							1 MM. APPLICATOR						
TIME	α		β		γ		δ	α		β		γ		δ
	Area	Blocked	Area	Blocked	Area	Blocked		Area	Blocked	Area	Blocked	Area	Blocked	
sec.	*per cent*	*per cent*	*per cent of* α	*per cent*	*per cent of* α	*per cent*		*per cent*	*per cent*	*per cent of* α	*per cent*	*per cent of* α	*per cent*	
0	100		41.1		41.6			100		33.1		31.6		
61														
3 mm.	93.8	6.2	31.8	23	19.0	33	Almost gone	101	0	31.6	4.5	31.6	0	Some gone?
63														
1 mm.														
103	89.4	10.6	18.6	55	9.8	76.5	Gone?	101	0	30.9	6.6	28.7	9.2	Some gone
234	64.2	35.8	15.5	62.2	All?	100?	100?	94.1	5.9	29.4	11.2	15.4	51.3	Many gone

What is the mechanism of the action on conduction? Since it has been demonstrated that electrical currents stimulate medullated fibers only via nodes of Ranvier the core conductor hypothesis of Hermann can stand only provided propagation is saltatory. The experiments of Erlanger and Blair (1934) indicate that progress may be saltatory and these authors also have shown in support of the core conductor hypothesis, that a blocked impulse raises the excitability at points some distance in advance of the block (1936). But Kato *et al.* describe an experiment through which they believe they have excluded the possibility of external eddy currents and yet the fiber continued to conduct. In their experiments an internode, held high and dry, bridged two pools of Ringer's in which the nodes were kept moist. They do not mention having measured the resistance between the two pools, although they did perform a control experiment in which they showed that a dried, but conducting internode could be blocked by

local cooling. It is possible, however, that cooling so alters the electrical properties of the cooled segment as to diminish its electrical response.

It must be borne in mind in such experiments that the axis cylinder of an internode of a 20 *micron* fiber has a calculated resistance of the order of 30 megohms. Such a high resistance should cause the stimulating effect of the extrinsic current to be independent, within wide limits, of the resistance of the external pathway. That there is, at least, a certain amount of independence is demonstrated by our experiments in which an internode was treated with glucose solution (for 16 min., to be specific) without interfering with nerve conduction, that is, if the slight notch appearing on the rising phase of the spike (fig. 1, *K*) can be ascribed to diffusion.

Some conception of the salt concentration, at the end of 16 minutes, in the disk of "nerve space" through which the internode in question passed can be gained from experiments on electrical conductivity which we have performed in this connection. It is not possible to go into the details of these experiments. Suffice it to say that at the time a root, treated with glucose solution, fails to conduct, electrical measurements indicate that the "nerve space" has a conductivity that is much less than that of half Ringer's solution. Since 4 to 7 seconds suffice to block the nerve impulse under these conditions, it is obvious that (barring such diffusion as may occur from adjacent untreated nerve or (and) from the nodes of adjacent fibers) the nerve space after 16 minutes of glucose treatment must have a very low salt concentration and a correspondingly low electrical conductivity. To be sure, external eddy currents still can flow through adjacent fibers. But the resistance in the total path must be materially increased (though, naturally, not nearly to that of the fiber segment) and yet there is associated with this increase little or no evidence of interference with nerve propagation. In the experiment of the Japanese investigators a resistance of several megohms between the pools probably would not have sufficed to interfere with the propagation of the nerve impulse; a thin, invisible film of moisture connecting the broad pools could have supplied an adequate path.

If impulse propagation is by an electrical process, block resulting from treatment of the nerve with glucose solution might result from 1, increased electrical resistance, 2, decreased spike height, or 3, decreased excitability. 1. Evidence has just been presented indicating that the increase in resistance does not suffice to account for the block; 2, it has been shown that though the recorded spike height does diminish as block develops, the diminution is only apparent; 3, to account for the block there is left, then, decrease in excitability of the axon. Direct observation shows that the excitability of the fiber actually is decreased by the glucose solution.

To determine the effect of the solution on excitability it is necessary to

expose the stimulated region to the solution and that, of course, alters the local resistance. If it can be assumed that prior to the development of block there is no appreciable change in the resistance of the axon but only in the medium surrounding the axon, the safest procedure in determining threshold changes must be to employ a stimulating circuit such that the voltage is independent of changes in the nerve resistance rather than one in which the current is independent. Determinations made by the former method show that the threshold of the most irritable fiber is raised when the root is treated locally with glucose solution.

To give the data of one of the experiments, treatment of the stimulated part of a root with a mixture of isotonic glucose solution (90 parts) and Ringer's solution (10 parts), a combination which may not block conduction in hours (the nature of the experiment precludes the use of a blocking concentration), raised the threshold to 1.3 times the normal value in less than 5 minutes. Subsequent treatment with full Ringer's solution brought the excitability back to normal in less than $3\frac{1}{2}$ minutes. That the changes in electrode contact resistance introduced an insignificant error is indicated by the fact that the "threshold" voltage did not rise significantly when the nerve was treated with the glucose solution until about $2\frac{1}{2}$ minutes had elapsed, and that then in less than two minutes the threshold rose to a plateau that remained reasonably level throughout the remainder of the period (about 3 min.).

It is generally believed that the physiological effects of "inert" isotonic solutions are exerted through action on the "surface-membranes of cells" (Lillie, 1932). Recently Fenn *et al.* have presented evidence which we interpret to mean that a potassium shift occurs fairly readily between nerve fibers and a surrounding medium. No attempt was made by these investigators to ascertain the physiological state of the fibers when the exchanges take place. The present results seem to fit best the view that the changes in the reactivity of the fibers are dependent on changes in their composition.

1. Small fibers block, in general, before large. In many experiments with multifiber responses we have been able to demonstrate blocking of all of the small (*delta*) fibers at stages when many, indeed most, of the larger fibers still were conducting. It is evident, therefore, that a salt atmosphere that blocks small fibers may not suffice to block large fibers. It seems probable, therefore, that the earlier incapacitation of small fibers is referable to faster depletion of salt content permitted by their smaller surface-volume relationship.

2. Large fibers recover from the effect of glucose treatment earlier, though all of the fibers are exposed to the same salt atmosphere.

3. Under glucose treatment small fibers reach the stage much earlier than large where treatment with Ringer's solution fails to effect recovery.

Apparently they have been deprived of something they are not in a position to replace.

4. Isotonic NaCl may effect the recovery of fibers that have failed in this manner. This and other casual observations indicate that the fiber that has failed behaves as if it were Ca sensitive. Such behavior might be the expression of K loss.

5. Under treatment with the solution the height of the extrinsic potential changes in such a manner as to indicate that up to the time of block, the core resistance does not increase materially; that later it mounts rapidly for a while and then more slowly. Such a sequence finds a ready explanation in depletion of the core of salts.

It is of interest to add that the pictures produced by the action of cocaine (Gasser and Erlanger, 1929) and of glucose solution are very much alike. Moreover, neither cocaine (Bishop, 1932) nor glucose solution decreases spike height as it blocks. Both block by reducing excitability. And it is now known that cocaine (Kato *et al.*, 1936, 1938), like glucose solution, produces its effects via the nodes of Ranvier. The vagaries in the responses to treatment with both procedures presumably are referable to structural variations of the nodes.

SUMMARY

1. Conduction in small spinal roots of the bullfrog is blocked by a few seconds of treatment with isotonic glucose solution.

2. The block, if not too long maintained, is reasonably reversible under treatment with Ringer's solution.

3. By recording the action potential of single fibers in a root it can be shown that topical application of the glucose solution to short (1 mm.) stretches of the root blocks only at definite loci, usually 2 to 3 mm. apart. The block may develop within a few seconds. Applied between such loci the glucose solution fails to block within any reasonable time. The loci are regarded as nodes of Ranvier.

4. When such a locus of least resistance is close to the recording electrode there is an *apparent* slight reduction in axon spike height as block develops, but the "extrinsic potential" maintains its full height. If the treatment be continued, the height of the extrinsic potential then falls and at a decelerating rate.

5. This and other evidence indicates that reduction of electrical conductivity of the "nerve space" cannot be a significant factor in the production of the block.

6. It is shown that block by glucose solution is due to reduced excitability. Block from excessive treatment with glucose cannot be reversed with Ringer's solution, but can be reversed by treatment of the depressed locus with isotonic NaCl.

7. In large roots, with all or many of the fibers responding, the glucose solution blocks the small fibers in general before it blocks the large. Naturally, a narrow (1 mm.) application blocks much more slowly than a wide (9 mm.) one, but the relative rate of action on large as compared with small fibers is the same under both treatments.

8. In small roots, with relatively few fibers responding, narrow application seems to favor slightly the blocking of small fibers, while wide application, if anything, favors the blocking of large fibers, a result that one would anticipate, not only in small but also in large roots, on the basis of the relation between segment length and fiber size.

9. Whether large fibers block first or last they always are the first to recover. Small fibers always are the first to fail irreversibly under repeated or prolonged treatment.

10. The evidence indicates that the glucose solution produces its effects, not only by changing the composition of the "nerve space" but also, and particularly after block has developed, by changing the salt composition of the fiber. A fiber that fails to recover in glucose solution usually acts as if it were Ca sensitive. It is suggested that this is due to loss of K.

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THE RELATION BETWEEN THE OXYGENATION OF FLUIDS AND THE OCCURRENCE OF EDEMA IN THE PERFUSED FROG WEB

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In a preliminary notice, it was reported that frogs can be perfused for five to six hours, without the occurrence of edema, if a fluid containing ox red cells and acacia as colloid is used (Saslow, 1936). Fluids lacking colloid, red cells, or both colloid and red cells are equally ineffective. If we accept Starling's hypothesis of fluid exchange through capillary endothelium, a plausible explanation of the efficacy of the acacia-red cell fluid is that adequate oxygenation permits the capillary endothelium to remain impermeable to the acacia for a relatively long time.

The present paper describes the original experiments and others bearing upon the explanation mentioned.

METHOD. The frogs were pithed completely before being cannulated. Various procedures were used in the preparation and cannulation without noticeable difference in result. In most of the experiments performed in 1936, the inflow cannula was placed in the sinus venosus, and the perfusion fluid left the animal from the cut post-cava and the two popliteal veins; in the majority of those performed in 1937, the fluid escaped from the cannulated median abdominal vein. The skin of both legs was prepared as described by Drinker (1927), so that the fluid could not short-circuit the web; escape of transudate was possible at the ankles. The web was perfused under the pressure of the heart beat, usually nearly maximal for each frog. The knees were supported with plasticine so that the web lay flat, and the web was spread over a glass slide on which plasticine held the digits fixed. The vessels tied off in the 1937 experiments were the coeliac artery, one aortic arch, and the pre-cavae; in female frogs, the ovarian pedicles were ligated. No vessels were tied off in the 1936 experiments.

The development of edema in the web was followed generally by keeping a microscope focussed upon an easily distinguishable melanophore. The adjustment had to be turned through about 0.03 mm. to produce noticeable differences in focus at the magnification used (120 ×). This method of detecting early edema was described by Drinker. I have observed as has he that once edema so observed sets in, it progresses rapidly and is

recognizable grossly in from 10 to 20 minutes from the first indication of web swelling at a magnification of 120 ×. Since the differences between the various solutions used are a matter of hours, the advantage of the more sensitive method is small. It is, however, less subjective. In a few experiments edema was observed grossly only.

The uniformity of the condition of the web during an experiment was estimated as follows. In all experiments one horizontal limb of the inflow cannula was connected with a reservoir of a perfusion fluid containing ox red cells. The other horizontal limb conducted into the animal a fluid without red cells. The vascular system was always cleared of frog red cells by a 5 to 15 minutes' perfusion with the cell-free fluid, then the red cell fluid was run in. The time which elapsed from the moment of entrance of the red cell fluid into the ventricle to the appearance of ox red cells in the microscopically observed capillaries was taken as an indication of the condition of the web. This "appearance time" was measured at intervals during an experiment. The method is similar to that of Drinker, who used a graphite perfusion fluid as indicator. A steady "appearance time" presumably means that the manipulations necessary during an experiment have produced no disturbance in the circulatory system as a whole.

Perfusion rates were obtained from time to time during an experiment by measuring the volume of fluid collected as it dripped from the curved frog board.

The completeness with which a given perfusion fluid replaced the frog's blood was determined by testing a centrifuged sample of perfusate (an acacia-red cell solution) with concentrated nitric acid (Heller's ring test). The test was still positive with frog serum diluted from 800 to 900 times, but negative at greater dilutions. A negative test signifies that not more than about 0.002 per cent of frog serum protein can be present in the perfusate, since the serum protein concentration in some of the frogs used was found to be of the order of 2 per cent. In three experiments designed to study the point, the centrifuged perfusate gave a negative test after 10, 30, and 45 minutes of perfusion at rates of from 100 to 130 cc. per hour. We may, then, consider the concentration of frog serum protein in the perfusing fluid to be about 0.002 per cent or less after from 10 to 45 minutes; similarly, all other frog serum constituents are diluted 800 to 900 times or more.

The blood used in the experiments was defibrinated ox blood which had been kept at 4° to 6°C. from 1 to 5 days. When only the red cells were used, they were washed three times in buffered Ringer-Locke's solution.

The perfusion fluids used had the composition and properties shown as follows:

1. Ringer's solution.—NaCl 0.65, KCl 0.014, $CaCl_2$ 0.012 per cent; P(NaH_2PO_4 and Na_2HPO_4) 9 to 10 mgm. per cent; pH 7.3 by indicator.

2. Acacia (3 per cent)-Ringer's solution.—The sterilized 30 per cent solution (without NaCl) of Eli Lilly and Company was used. In the 1936 experiments, the samples were from various lots; in the 1937 ones, all samples were prepared for us by Eli Lilly and Company from one large batch of the dried gum. Calcium chloride was omitted from the Ringer's solution when acacia was present, since the calcium content of 3 per cent acacia in water is from 0.0126 to 0.0136 per cent.[1] Na, K, and P were added as for Ringer's solution. The pH of the acacia-Ringer's was 7.2 by indicator and by quinhydrone electrode. The colloid osmotic pressure of the solution was determined to be 105 mm. H_2O, by the second method of Krogh and Nakazawa (1927).

3. Acacia (3 per cent)-ox red cell-Ringer's solution.—Washed ox red cells were added to the acacia-Ringer's solution so that the percentage volume lay between 16 and 30. The acacia was added to this perfusion fluid just before it was to be used; I have never observed any agglutination of the red cells during even a 5 or 6 hour perfusion. The pH of the fluid after centrifugalization was 7.3 by quinhydrone electrode. The oxygen capacity of samples of this fluid made up on different days with the percentage volume of red cells equal to 21 varied from 8.5 to 9.0 volumes per cent. This is approximately the same as the oxygen capacity of the blood of various species of *Rana* (McCutcheon, 1936); there seem to be no data on the oxygen capacity of the blood of *Rana pipiens*.

4. Acacia (3 per cent)-serum-Ringer's solution.—Ox serum or horse serum was added to acacia-Ringer's solution. The pH was 7.3 by quinhydrone electrode. The oxygen capacity of the solution acacia (3 per cent)-horse serum (20 per cent)-Ringer's at 19°C. and pO_2 749 mm. Hg was 2.65 volumes per cent; the oxygen content of the solution oxygenated vigorously in the perfusion reservoir at 19°C. and barometric pressure 769 mm. Hg while leaving the reservoir to enter the inflow cannula at 250 cc. per hour was 2.30 volumes per cent. This solution was therefore 87 per cent saturated throughout an experiment. The colloidal osmotic pressure of the solution with 20 per cent horse serum was 147 mm. H_2O. The horse serum samples were part of a large lot of normal horse serum prepared by the Antitoxin and Vaccine Laboratory, Massachusetts State Department of Public Health, Boston. The serum was sterilized by filtration through a Berkefeld candle, put up without preservative in 50 cc. bottles and kept at 4° to 6°C. until used.

The perfusion fluids were oxygenated by bubbling tank oxygen through them continuously.

[1] The inorganic composition of the 30 per cent acacia is stated by Eli Lilly and Company to be: Ca 0.126–0.136 per cent, Mg 0.0015–0.0038 per cent, PO_4 none, heavy metals none by U. S. P. XI test, pH 5.0–5.2.

The experiments were performed at temperatures between 21° and 24°C., except for those in the pressure chamber, where the temperature was 25° or 26°C.

R. pipiens both male and female were used throughout. The specimens weighed from 40 to 80 grams.

About half the experiments were performed at Woods Hole in August and September, 1936, and the rest in Boston between September, 1937, and January, 1938.

RESULTS. The results of typical perfusion experiments are summarized in table 1. These experiments show that it is possible to perfuse frogs for several hours without even incipient edema if the colloid osmotic pressure of the fluid is adequate[2] and if it contains a sufficient number of ox red cells.

It is possible to suppose that the effectiveness of the red cell-acacia fluid is due to a combination or to any one of several related factors other than the characteristic which at once suggests itself, namely, the high oxygen content of the fluid. These factors are increased viscosity (as compared with the other fluids), decreased arterial and capillary pressures, decreased heart rate, and decreased volume perfused per unit of time. Singly or in combination these factors could lead to decreased filtration through the capillaries. They are considered briefly here.

Viscosity. Went (1928) was unable to detect any difference in the viscosity of acacia-Ringer's solutions without and with mammalian red cells, up to 50 per cent by volume.

Arterial pressure. This was measured in the sciatic artery in several experiments in which a given frog was perfused with a series of fluids. The solutions compared were red cells-acacia-Ringer's, acacia-Ringer's, acacia-horse serum-Ringer's, and red cells-horse serum (20 per cent)—Ringer's. The pressures were read with the degree of filling of the heart as nearly the same as could be judged by eye, that is, nearly maximal. The shifts in arterial pressure produced by changing from one of these fluids to another did not exceed 3 mm. Hg and occurred in no consistent manner.

Heart rate and perfusion rate. In similar experiments, heart rate and perfusion rate did not show significant changes as one fluid replaced another.

Capillary pressure. This was not measured. But with steady heart rate, perfusion rate and arterial pressure it is hardly likely that the general capillary pressure changed noticeably as one fluid replaced another.

We return, then, to a consideration of the most plausible explanation of the effectiveness of the red cell-acacia-Ringer's. Even if it is granted

[2] The colloid osmotic pressure of frog serum has been reported as being in the range of 55 to 134 mm. H_2O (Churchill, Nakazawa and Drinker, 1927; Krogh, 1922; Landis, 1927; White, 1924). The colloid osmotic pressure of the horse serum used was 250 mm. H_2O; that of the ox serum was not determined.

that this may be its oxygen content, it has still to be shown that the oxygen is actually available. The red cells are mammalian, and the temperature coefficient of the dissociation of one kind of mammalian oxyhemoglobin (human) is known to be high (Brown and Hill, 1923); it is necessary to determine whether ox HbO_2 dissociates appreciably at the temperatures

TABLE 1

Edema and composition of perfusion fluid

PERFUSION FLUID	TIME REQUIRED FOR APPEARANCE OF EDEMA		APPEARANCE TIME AT		PERFUSION RATE	NUMBER OF EXPERIMENTS
	120 ×	Grossly	Start	End		
	min.	*min.*	*sec.*	*sec.*	*cc./hr.*	
Ringer's	15–50	30– 60	10–30	10–22	80–150	11
Ringer's 3 per cent acacia	20–35	25– 45	8–15	10–40	80–200	5
Ringer's 3 per cent acacia 20 per cent ox serum	10–45	20– 55	10–30	15–60	120–150	4
Ringer's 3 per cent acacia 40 per cent ox serum	15	25	17	17	120	1
Ringer's 3 per cent acacia 20 per cent horse serum	20–35	25– 55	10–20	10–15	130–210	5
Ringer's 21–25 per cent ox red cells	20–50	25–140	5–10	10	150–200	4
Ringer's 19–24 per cent ox red cells 3 per cent acacia	No edema in experiments terminated in 3 to 6 hours		10–20	15–25	80–150	8
Ringer's 16 per cent ox red cells 29 per cent ox serum	No edema in experiments terminated in 2½ to 4 hours		15–20	15–25	90–130	5

and under the other conditions of these experiments. Determinations of oxygen content showed that the inflowing fluid had 8.5 to 9.0 volumes per cent (percentage volume = 21) and the outgoing fluid 3 to 6 volumes per cent. The ox HbO_2 was then 33 to 67 per cent desaturated, in different experiments.

The desaturation of ox HbO_2 which occurs increases simultaneously the CO_2 carrying power of the perfusion fluid. The smaller fall in pH which then would take place as the fluid circulates might be considered the important characteristic rather than the available oxygen as such. Against this consideration are the following facts. Capillaries seem insensitive to slight changes in pH of the fluid bathing them, over the range of pH 4 to 8 (Landis, 1934; Krogh, 1929; Atzler and Lehmann, 1922). Further, experiment showed that an inflowing and outgoing fluid not containing red cells, namely, acacia-Ringer's, did not differ by more than 0.1 pH unit during a perfusion. It must be concluded that the fluids not containing red cells are nevertheless adequately buffered, and that any slight increase in the buffering capacity of the fluid with red cells as a consequence of desaturation is not the factor making it effective.

The possibility should be considered that ox serum constituents carried over to the red cell fluid with the washed cells play a rôle in preventing edema. These constituents are diluted more than 100,000 times by the time the cells have been added to the acacia-Ringer's, even if we assume that 10 per cent of the "packed cells" are supernatant fluid which remains each time with the cells after they have been centrifuged at 2500 r.p.m. for 30 minutes. Since 40 per cent ox serum-Ringer's does not prevent edema, this possibility amounts to considering the importance of some serum constituent (from ox or frog) present in high dilution and effective only in a fluid of high available oxygen content, for example, pituitrin.

That such a serum constituent (or a diffusible ox red cell constituent acting similarly) does or does not play a rôle could be determined only by performing experiments with a perfusion fluid containing no red cells but otherwise adequate. Such experiments were attempted in a pressure chamber which was available at the Harvard School of Public Health.

The perfusion fluid (acacia-Ringer's), in a small spirometer communicating with the air of a compartment of the chamber through a water seal, was oxygenated vigorously from a tank for $1\frac{1}{2}$ hours; during this time, the pressure in the compartment was either 45 or 60 lb. absolute. In this way, the oxygen content of the fluid could be raised to 9.3 volumes per cent when the pressure was 60 lb. While the fluid was being oxygenated, the frog was prepared and cannulated in another compartment at 15 lb. Perfusion was started with red cell-acacia-Ringer's and continued with it as the pressure in this compartment rose on signal to 45 or 60 lb., and then until acacia-Ringer's was started. An unfortunate feature of the experiments was that as the pressure rose in the chamber so did the temperature; the result was that for 40 to 60 minutes from the time the signal to compress was given, the temperature stayed between 28° and 31°C. After that, it remained steady at 25° or 26°C. for the rest of the experiment. The effects of the rise in temperature were reduced as much as possible by

wrapping the frog in absorbent paper soaked in cooled Ringer's, and by starting the acacia-Ringer's solution only when the temperature had reached its final steady value. The acacia-Ringer's was oxygenated continuously during the perfusion. Two experiments were carried out as described above. In a third, a red cell-acacia-Ringer's solution was used as perfusion fluid throughout; its oxygen content was 15.9 volumes per cent at a pressure of 60 lb. The method used for collecting samples of fluid and passing them out of the pressure chamber for analysis of oxygen content is that described by Behnke, Shaw, Shilling, Thomson, and Messer (1934).

The experiments (table 2) appear to show that acacia-Ringer's oxygenated under pressure is at least as effective in preventing edema as similarly treated red cell-acacia-Ringer's, and that therefore the important feature of the latter is its high content of available oxygen. The experiments are unavoidably few, because of risk to the operator, and involve also a reduction in the effectiveness of the red cell-acacia-Ringer's fluid

TABLE 2

Perfusion with fluids oxygenated under pressure

PERFUSION FLUID	FROG		TEMPERATURE	TIME TO APPEARANCE OF EDEMA		PERFUSION RATE	PRESSURE
	Sex	Weight		Microscopically	Grossly		
		gm.	*°C.*	*min.*	*min.*	*cc./hr.*	*lb. absolute*
Acacia-Ringer's	♀	82	26	90	100		45
Acacia-Ringer's	♀	84	25	85	90	250	60
Red cell-acacia-Ringer's	♀	74	26	50	60	180	60

as compared with its behavior at 15 lb. This reduction is possibly due to a combination of the rather high temperature for frogs, a factor in itself increasing transudation, and the fact that by the time the test fluid could be started, the animal had already been perfused for 50 to 80 minutes with red cell-acacia-Ringer's; for some 40 to 60 minutes of this period the red cell-acacia-Ringer's was being oxygenated under pressure and contained an unusually large amount (up to 9.3 volumes per cent) of physically dissolved oxygen. It is conceivable that such conditions may lead to a generalized poisoning of the frog capillary endothelium, resembling the oxygen poisoning of mammalian pulmonary endothelium (Smith, Bennett, Heim, Thomson, and Drinker, 1932).

Discussion. The conclusions drawn from the experiments described, namely, that the red cell-acacia-Ringer's solution prevents the early appearance of edema in perfusions and that it is effective because of its high content in available oxygen, are consistent with the observations of others. For example, Krogh (1929) was able to perfuse frogs with acacia

(3 per cent)-red cells (33 per cent)-Ringer's for several hours without edema, although the capillaries were dilated throughout. This dilatation of capillaries has also been observed in all the experiments with red cell-acacia-Ringer's reported here.

The sensitivity of frog capillary endothelium to the oxygen content of the fluid bathing it is shown in some experiments of Landis (1927–1928). When oxygen-free Ringer's was dropped upon exposed blood-containing capillaries of the mesentery, the filtration rate increased four times after 3 minutes.

It is doubtful that any rôle can be assigned to pituitrin in such experiments as those described here. Krogh (1929) supposed that pituitrin delayed the sudden relaxation of capillaries which preceded a markedly increased transudation; but Drinker (1927), working in the same laboratory, concluded that pituitrin did not retard the appearance of edema. Krogh's perfusion fluid contained ox red cells (33 per cent by volume); Drinker's did not. Krogh observed that with or without added pituitrin, but with red cells, perfusions of several hours' duration were possible. Without pituitrin the capillaries were dilated throughout. With pituitrin they were narrow. Apart from this difference, which seems irrelevant to the question of increased filtration (Landis, 1927), no others were demonstrated. So far I have not observed any difference in the duration of perfusion without edema whether red cell-acacia-Ringer's or red cell-serum-Ringer's is used; the capillaries are always dilated in the first case, and narrow (perhaps because of the presence of adequate pituitrin) in the second. The present experiments suggest that the effectiveness of Krogh's perfusion fluid was due to the red cells and not to the pituitrin.

The experiments of Drinker, in which acacia (3 per cent)-horse serum (20 per cent)-Ringer's was found to be effective in preventing edema, have still received no satisfactory explanation. I have been unable to repeat them. On two occasions, such a solution prevented the appearance of edema for from 75 to 85 minutes but never for periods comparable with those when red cells were added.

In general, the experiments reported here show that the red cell-acacia-Ringer's solution is as satisfactory in the perfusion of frogs as Stanbury, Warweg and Amberson (1936) have found it to be in total plasmapheresis experiments on mammals.

I am greatly indebted to Dr. Cecil K. Drinker for various suggestions made during the course of these experiments.

SUMMARY

1. The solution acacia (3 per cent)-ox red cells (19 to 24 per cent)-Ringer's can be perfused through frogs for periods up to 6 hours without the appearance of microscopically detectable edema.

2. The effectiveness of the solution appears to be due to its high content in available oxygen.

3. In such experiments, the frog serum proteins can be replaced by acacia having approximately the same colloid osmotic pressure.

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THE EFFECTS OF ADMINISTERING LARGE AMOUNTS OF CORTIN ON THE ADRENAL CORTICES OF NORMAL AND HYPOPHYSECTOMIZED RATS

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It has been observed by Ingle, Higgins and Kendall that atrophy of the adrenal cortices of male rats occurs during treatment with massive doses of cortin. The simultaneous administration of the adrenotropic principle of the anterior lobe of the pituitary body was effective in preventing the regression of the adrenal cortex which follows treatment with cortin. This observation led Kendall and me to postulate that the effect of treatment with cortin is mediated through the anterior lobe of the pituitary body which may inhibit its adrenotropic activity in the presence of an excess of cortin. This hypothesis has further support in the established facts that a similar atrophy of the adrenal cortices occurs following removal of the anterior lobe of the pituitary body, that the adrenal cortices are repaired when the hypophysectomized animal is treated with the adrenotropic principle and that the adrenal cortex fails to show any degree of hyperplasia when the hypophysectomized animal is subjected to severe forms of stress.

The object of the present experiment was to determine whether or not treatment of the rat with massive doses of cortin would influence the adrenal cortices if the level of adrenotropic principle in the body were held constant. This was attained by removing the pituitary body and substituting for it a constant intake of adrenotropic principle in an amount which was known to be adequate to maintain the adrenal cortices of hypophysectomized rats at a normal size.

METHODS. Sixty male rats each having an initial body weight of 180 grams were used. Ten rats were hypophysectomized but did not receive treatment; ten normal rats had their food intake restricted to the level voluntarily adopted by the untreated hypophysectomized animals; ten normal animals had no dietary restriction; ten normal animals received 10 cc. of cortin daily in their drinking water (a slight voluntary reduction in the intake of food was noted); ten hypophysectomized rats received 0.5

cc. of adrenotropic hormone[1] by intraperitoneal injection daily and 10 cc. of cortin daily in the drinking water, and ten additional hypophysectomized animals received 0.5 cc. of adrenotropic hormone daily but no cortin was administered. The food intake of the hypophysectomized rats which

TABLE 1

Effect of massive amounts of cortin on the body weight of normal rats and hypophysectomized rats treated with the adrenotropic hormone (one week)

EXPERIMENTAL CONDITION	NUMBER	BODY WEIGHT	
		Average	Range
		grams	*grams*
Normal; restricted diet; no treatment	10	141	134–149
Hypophysectomy; no treatment; voluntary restriction of diet	10	142.9	138–151
Normal; no restriction of diet; no cortin administered	10	196.4	184–210
Normal; 10 cc. of cortin given daily; slight voluntary restriction of diet	10	151.7	142–162
Hypophysectomy; 0.5 cc. of adrenotropic principle administered daily; no cortin administered; restriction of diet	10	141.4	136–147
Hypophysectomy; 0.5 cc. of adrenotropic principle administered daily; 10 cc. of cortin given daily; voluntary restriction of diet	10	137.7	125–143

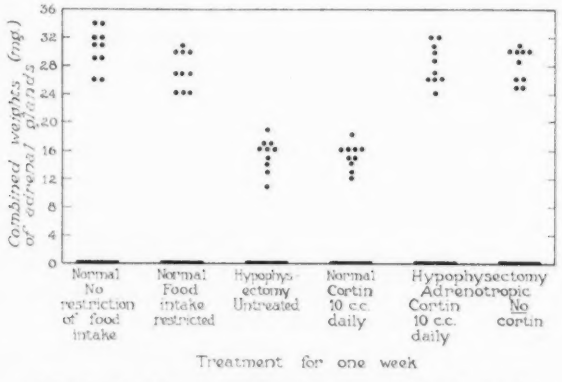

Fig. 1. Combined weights of pairs of adrenal glands

received the adrenotropic hormone was regulated in order to equate the loss of weight. To accomplish this it was necessary to restrict the food intake

[1] The adrenotropic hormone was prepared by H. D. Moon, of the Institute of Experimental Biology of the University of California, and was supplied to me through his courtesy.

of the rats which did not receive cortin to a definitely smaller amount than was consumed by the rats which did receive cortin. At the end of seven days all of the animals were killed and necropsy was performed.

RESULTS. The data on body weights are presented in table 1 and the data on adrenal weights are shown in figure 1.

It is clear that the administration of large amounts of cortin to the male rat produces a marked atrophy of the adrenal glands. On the contrary, when the anterior lobe of the pituitary body is absent and the animal receives adrenotropic hormone in amounts adequate to prevent the atrophy occurring from hypophysectomy alone, there is no apparent effect of cortin on the adrenal cortex. There is a definite tendency for the cortin-treated animals to lose in general body weight. Extensive atrophy of the thymus of those animals which received cortin was noted, thus confirming our previous observations (1, 3).

COMMENT. It is evident from these experiments that the administration of massive amounts of cortin to the rat does not injure the adrenal cortices directly. The hypothesis that atrophy of the adrenal cortex may be due to restriction of the output of the adrenotropic principle of the anterior lobe of the pituitary body is supported by these observations.

The loss in adrenal weight is essentially cortical as we have shown. This loss in mass of cortex does not accompany a loss in total body weight which is induced by the restriction of food. It also has been observed by Dr. L. T. Samuels, Department of Physiology, University of Minnesota, that when the total body weight of the hypophysectomized rat is sustained at its preoperative level by forced feeding the adrenal cortices still undergo atrophy. The loss of volume of the adrenal cortex which occurs following the administration of cortin or following the removal of the anterior lobe of the pituitary body is probably causally independent of the concomitant loss in total body weight.

SUMMARY

When normal rats are treated with massive amounts of cortin an extensive atrophy of the adrenal cortices consistently occurs. When the pituitary body is absent and the animal receives adrenotropic hormone in constant amounts there is no apparent effect of cortin on the adrenal cortex.

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THE EFFECT OF ACETYLCHOLINE ON THE EXCITABILITY OF THE FROG'S SARTORIUS MUSCLE

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If, as many now believe, (e.g., Dale, Feldberg and Vogt, 1936; Brown, Dale and Feldberg, 1937) acetylcholine is released at the endings of motor nerves for the purpose of exciting the muscle, it is to be expected perhaps that muscles dipped into acetylcholine solutions too weak to cause contractions would show changes in excitability to electrical stimuli. For it seems to be true generally, that stimuli of different kinds are additive in their effects, i.e., that electrical stimuli aid mechanical or chemical stimuli and so on. Experiments were done, therefore, to determine the effect of acetylcholine on the α strength duration curve of the frog's (Rana pipiens) sartorius muscle.

METHOD. The muscle was usually dissected the day previous to the experiment and kept in cold Ringer's solution. For the measurements it was suspended in Ringer's in a glass tube with a constricted portion in which the pelvic end was placed so that the current density was much higher there than in the remainder of the muscle (Blair, 1938). Care was taken to avoid disturbing the position of muscle when the solution in the tube was changed in order to add reagents in Ringer's. The strength-duration curve was measured with direct currents of various durations from 4 to 112 msec. by means of an apparatus described previously (Blair, 1935). Usually two initial strength-duration curves were taken at an interval of about one hour with the muscle in Ringer's solution to make sure that the preparation was stable before further measurements were made. A minimal contraction of the muscle at a distance from the point of stimulation was used as the index of an effective stimulus.

The acetylcholine in all cases was made up as a 0.1 per cent solution of acetylcholine chloride in 5 per cent acid sodium phosphate. This was diluted by Ringer's solution to the extent required just before using.

Eserine solutions were made by diluting with Ringer's stock solution of 2 mgm. eserine sulphate with 1 gram boric acid in 1 litre of water.

The Ringer's solution contained 0.65 gram NaCl, 0.01 gram KCl, 0.015 gram $CaCl_2$, 0.192 gram Na_2HPO_4 ($12H_2O$) and 0.0184 gram $NaHPO_4$ (H_2O) per 100 cc.

EXPERIMENTAL RESULTS. It soon became evident that the results with acetylcholine divided themselves into two classes, those in which the concentration was too weak to cause a period of twitching of the muscle and those in which twitching occurred. In the former case the acetylcholine appeared to have no effect whatsoever on the chronaxie of the muscle or the rheobase. In the latter case there were marked changes of both.

In figure 1 are plotted the rheobases of both the muscle and the nerve for a series of increasing concentrations of acetylcholine. The measurement of the two rheobases was made possible by placing the pelvic end of the muscle a little farther than usually into the constricted portion of

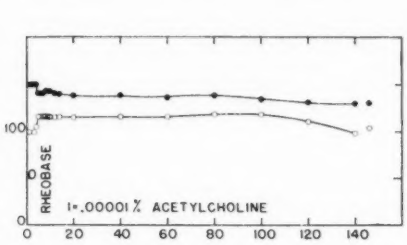

Fig. 1

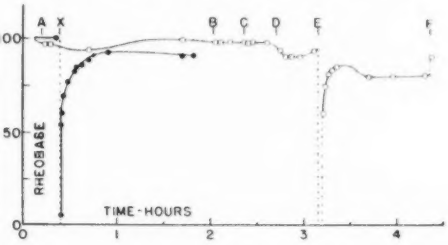

Fig. 2

Fig. 1. The nerve rheobase, dots, and the muscle rheobase, circles, with increasing concentrations of acetylcholine chloride in Ringer's solution. The concentration was changed by changing the solution. The last, the separated points, were taken after returning to Ringer's solution. The total time of the experiment was 3 hours.

Fig. 2. The circles show the muscle rheobase starting with a value 100 in Ringer's solution. The solution was changed at *A* to 0.00001 per cent acetylcholine, at *B* to 0.00005 per cent, at *C* to 0.0001 per cent, at *D* to Ringer's then back to 0.0001 per cent, at *E* to 0.0005 per cent acetylcholine. The last solution, only, caused the muscle to twitch. At *F* Ringer's was re-introduced. The dots show another muscle which responded by twitching to 0.00005 per cent acetylcholine at *X*.

the tube so as to increase the current density in the region of the nerve. In this particular case the placing was such that the nerve began to respond when the current was raised to about two muscle rheobases. It will be observed that apart from a sharp increase of the muscle and a decrease of the nerve rheobase at a low concentration 0.00005 per cent, which were probably due to a change of position of the muscle since they did not occur in other cases, the rheobases remained quite steady up to a concentration of 0.00140 per cent acetylcholine. Also, on returning to Ringer's solution no great change occurred as is indicated by the last, the isolated points on the diagram.

A similar case for the muscle rheobase alone is given in figure 2 by the

first part of the upper curve described with open circles. This time, however, on reaching a concentration of 0.0005 per cent acetylcholine, twitching of the muscle occurred. This lasted two minutes during which time the rheobase can be said to have been zero. Following the twitching the rheobase was low but it rose within a few minutes to about 80 per cent of its former value after which it sank slightly and remained steady. On going back to Ringer's the rheobase rose abruptly to about the same value as it had previously to the twitching.

Another case of the recovery of the rheobase after twitching had occurred with 0.00005 per cent acetylcholine is given by the dotted curve of figure 2. In this case the final value is not so low relative to the normal and in general the rheobase recovered more nearly to normal the lower

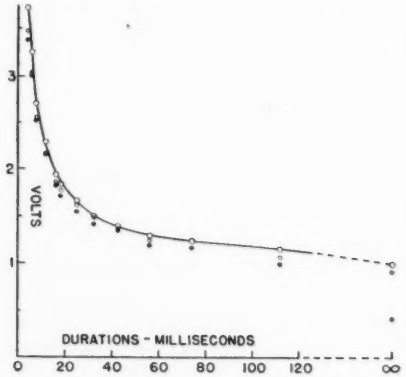

Fig. 3. α strength-duration curves; the large circles in Ringer's at 19°C., the dots 25 minutes after changing to 0.001 per cent acetylcholine solution, the small circles 10 minutes after returning to Ringer's.

the concentration of acetylcholine. Figure 3 gives an example of a greater lowering of the rheobase with a greater concentration than those of the examples in figure 2. In figure 3 are plotted three strength-duration curves of the same muscle, first in Ringer's, then about half an hour after going into 0.001 per cent acetylcholine, then finally on returning to Ringer's. With this strong concentration the rheobase recovered to somewhat less than half of its initial value after a period of twitching.

It will be observed also in figure 3 that the chronaxie in Ringer's both initially and finally is about 15 msec. while in the acetylcholine it is greater than 120 msec. An increased chronaxie is also quite general after twitching has occurred and the rheobase has recovered to a steady state. And as with the rheobase the change is usually less, the smaller the concentration.

As was remarked above, concentrations which do not cause twitching,

leave the chronaxie as well as the rheobase constant. This is illustrated by table 1 in which the chronaxies of the preparation of the upper curve of figure 2 are given. It will be observed that there is very little change until the reading at 3:46 which followed a period of twitching. Similar results were obtained in all the experiments.

It is not the absolute concentration of the acetylcholine which determines whether there shall be twitching followed by changes of the rheobase and chronaxie because, as a rule, a concentration two or three times as high can be reached without effect if it is approached by small steps than is reached directly. Another feature of the response to the drug which is probably important is that, whereas it requires about ten times as high a concentration to cause twitching at the nerve-free end of the muscle as it does in the region of entry of the nerve, in no case was there a failure of the rheobase and chronaxie to change after twitching. Thus it appears that the muscle is altered at its nerve free end even though the preceding and apparently requisite twitching is brought about by the action of the

TABLE 1

Time	0	0:17	1:42	2:13	3:0	3:46	4:25
Chronaxie	23.5	23.1	23.7	23.8	25.4	36.3	26.4
Solution	R	0.00001	0.00001	0.00005	0.0001	0.0005	R

The chronaxies in milliseconds of the muscle of figure 2 (circles) at various stages. R is in Ringer's solution. The numbers give the percentage of acetylcholine chloride solutions.

acetylcholine at the region of the nerve endings. On the other hand, twitching of the muscle is not of itself sufficient because it has no effect when it is elicited by electrical stimuli whether through the nerve or the muscle.

The effect of eserine. In a number of cases eserine sulphate 0.01 or 0.02 mgm. per litre was used with or without acetylcholine. The only change in the strength-curve observed with eserine by itself was a tendency for the readings to rise on frequent successive stimulation. Acetylcholine appeared to have the same effect on the rheobase and chronaxie with or without eserine. On returning to Ringer's from eserine alone or from eserine with acetylcholine the muscles tended to twitch spontaneously after a single stimulation. Thus the effect of eserine on the whole appeared to be one leading to a decrease of the stability of the preparation rather than giving more effectiveness to the acetylcholine.

Since the nerve-free part of the sartorius (Pezard and May, 1937) contains choline esterase, according to Marnay and Nachmansohn (1938) although in smaller quantities than the innervated part, it is to be expected perhaps that some disappearance of acetylcholine would occur even though the solution in the chamber was of relatively large volume about

50 cc. Also the drug is probably not altogether stable in the Ringer's solution used. The lowering of the rheobase and the lengthening of chronaxie showed no definite tendency to disappear, however, in preparations kept for 1 to 4 hours in acetylcholine alone before returning to Ringer's. It is not to be expected, therefore, that eserine would make any difference except perhaps at longer times.

The effect of curare. On applying acetylcholine locally to parts of a pair of matched muscles, one curarized, the curarized preparation required a somewhat higher concentration than the normal to cause twitching both at the nerve-containing and the nerve-free regions. Also, concentrations effective for twitching in both gave rise to a briefer response in the curarized muscle. Thus curarization does not prevent the response to acetylcholine but it necessitates stronger dosage for the same effect. This was not followed further since denervated mammalian muscle has been shown by Dale and Gasser (1926) and others to be sensitive to acetylcholine after curarization, although the response in this case may be contracture, at least in part. Also, although Brown (1937) found that curarine almost abolished the response to ordinarily effective doses of acetylcholine injected into the blood vessels, no doubt increased doses would be effective by this route although they would perhaps act directly on the muscle rather than the junctional substance.

The effect on the strength-duration curve of the addition of acetylcholine to a curarized muscle was tried on only one preparation. In this case the usual twitching and lowering of the rheobase and lengthening of chronaxie occurred. A high concentration, 0.005 per cent of acetylcholine, was used and no attempt was made to find the threshold.

Discussion of results. As was remarked above, stimuli of different kinds appear to assist each other. Tigerstedt (1881–82), for example, demonstrated Pflüger's laws, by testing with mechanical stimuli rather than electrical. In this case the state of excitation produced by the polarizing current can be measured equally well by mechanical stimuli indicating that both electrical and mechanical stimuli act through the same mechanism.

Potassium may be used as an example of a chemical stimulator. It gives rise, in fact, to reponses of muscle rather similar to those caused by acetylcholine. Potassium in concentrations too low to elicit mechanical responses shows a transient increase of sensitivity to electrical stimuli, i.e., a lowering of the rheobase of the α excitability of the frog's sartorius (Chao, 1937).

Thus acetylcholine may perhaps excite muscle through a different mechanism than electrical stimuli or through a process with a different initial stage. This stage whether at the region of the nerve ending or in

the muscle substance itself is set off to affect the ordinary excitation mechanism only by a definite threshold amount of acetylcholine, a lesser amount having no influence at all. When the threshold is reached, the muscle responds in the usual way as indicated by the action potential and the twitch, but in addition there is a change of condition made manifest by the lowering of the rheobase and the lengthening of chronaxie.

It does not appear from these results that acetylcholine released from the nerve endings could assist the action potential of the nerve to stimulate the muscle, unless the first discharge of acetylcholine were sufficiently great to stimulate by itself, or until sufficient had accumulated to elicit a response by itself. The phase following this and corresponding to the period of low rheobase, figure 2, which probably could be maintained by successive discharges and disappearances of acetylcholine, might very well be one in which facilitation could take place. Thus if there is both the occurrence of an action potential as the primary transmitting factor and the production of acetylcholine at the nerve ending it may be supposed that the function of the acetylcholine is that of sensitizing the muscle in order to keep it well within the range of the action current as continuous activation of the muscle by the nerve proceeds. Or if acetylcholine is the primary transmitter it appears inevitable that it will, after being once effective, facilitate stimulation by succeeding action currents even though they are secondary factors, unless they are much weaker stimuli than has been commonly supposed.

In any case, whether or not the action current is the primary factor in transmission it can scarcely be considered without further evidence not to be a secondary factor. If it is the primary factor it may appear on consideration that the phenomena ascribed to acetylcholine as a transmitter can equally well be ascribed to acetylcholine as a facilitator or sensitizer.

SUMMARY

A study of the effect of acetylcholine in Ringer's solution on the α strength-duration curve of the frog's sartorius muscle shows that the drug has no effect until a concentration is reached which causes twitching of the muscle. Following the twitching, the rheobase is very low but it recovers rapidly to a value below the normal by an amount which usually is greater, the higher the concentration of the acetylcholine. At this stage which persists for at least a few hours the chronaxie is considerably lengthened. On returning to Ringer's solution both the rheobase and chronaxie return quickly to normal. It is suggested that acetylcholine does not excite muscle through the same mechanism as do electrical stimuli.

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THE RESPONSE OF THE SMOOTH MUSCLE OF THE GALL BLADDER AT VARIOUS INTRAVESICAL PRESSURES TO CHOLECYSTOKININ

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This work was undertaken to obtain evidence bearing on the cause of the variable responses of the gall bladder to cholecystokinin. It has been observed previously: (*a*), that the gall bladder of a fed dog, or of one in which the intragall-bladder pressure is very high (20 cm. bile) does not respond well; (*b*), that, if in the process of cannulating the viscus for recording pressure changes considerable bile is spilled and not returned, the viscus does not respond well; (*c*), that if the intragall-bladder pressure is adjusted to 5 or 7 cm. of bile pressure, a good response is uniformly obtained; and (*d*), the latter pressure has been selected on the basis of experience as the optimum pressure for the assay of cholecystokinin. It was thought that if such variable responses could be explained, the explanation might be applicable in part to those human gall bladders which when distended with bile do not evacuate well in response to egg yolk and cream. Further, the gall bladder of a person may not evacuate well, and then at a later examination may evacuate well. These latter observations have been ascribed either to a hypertonic sphincter of Oddi, to a slowly evacuating stomach, to variations in the viscosity of bile and to a narrow cystic duct. A factor which has not been considered is that the variable responses may be due also to variations in intragall-bladder pressure. At least, if the smooth muscle of the gall bladder is like smooth muscle (1, 2, 3, 4) elsewhere, an optimum tension for maximal contraction should be demonstrable.

METHODS. In this study the isolated gall bladder of the guinea pig, and the gall bladder in situ of the dog were used.

In some of the experiments on the gall bladder of the guinea pig, the apparatus shown in figure 1 was used. The graduated glass tube *A* served as a manometer and the side arm *B* as a pressure regulating device. The gall bladder was removed from the guinea pig following decapitation.

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The apparatus was filled with Sollmann-Rademaeker's solution or S-R solution (6), and then the cannulated end of the apparatus was inserted into the cystic duct with as little spillage of bile as possible, none being spilled in most instances. The dome of the gall bladder was attached to a muscle lever. The gall bladder in place was then submerged in a bath containing 50 cc. of S-R solution at 40°C., the solution being aerated by the usual method. The apparatus shown in figure 1 was used only when we desired to study the longitudinal shortening of the gall bladder in response to cholecystokinin, or CCK. Otherwise we used an apparatus like that in figure 1, except that the cannulated end was not turned upwards, but pointed downwards so that the gall bladder was simply suspended in the bath. With this technique the response of the gall bladder to a standard submaximal dose of CCK could be determined at

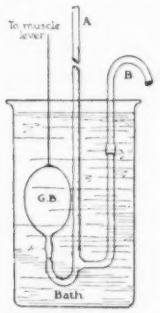

Fig. 1

different initial intravesical pressure levels. The total pressure increment could be read from the manometer.

In the experiments on the dog's gall bladder in situ, the method of Ivy and Oldberg (7) was used, except that a side arm for introducing or removing bile for the purpose of regulating intravesical pressure was attached to the cannula inserted into the dome of the gall bladder. The increment in pressure resulting from an injection of CCK was read from the manometer without the tambour attached.

In all instances the initial pressure was changed by altering the volume of fluid or bile in the system. The volume-pressure changes were made slowly, and adequate time was allowed for the musculature to become adjusted to its new volume-pressure relations. This sometimes required one-half hour, and in a number of instances, as will be noted below, adjustment did not result.

The animals were fasted for 24 hours before the experiment to insure a viscus well filled with bile. The initial volume and pressure of the viscus

was noted to take cognizance of those gall bladders which would not adjust readily to changes in their pressure-volume relationship, or which were "overstretched" as a result of high pressures. In the dogs the amount of bile introduced or removed was noted for the same reason.

A preparation of CCK was used which has been described previously (5). Three-tenths of a milligram constituted a dog unit of CCK and 0.1 mgm. a dog unit of secretin. A 0.1 mgm. portion of this preparation, when added to a 50 cc. bath, caused most guinea pig gall bladders to contract submaximally; five-tenths of a milligram caused the gall bladder of all dogs to contract submaximally. This dose was considerably less than that employed by Jung and Greengard (6), because our preparation was more soluble and concentrated.

RESULTS. *Does presence or absence of bile in gall bladder modify its responsiveness to CCK?* Whether the presence or absence of bile in the gall bladder influenced the excitability of the smooth muscle had to be determined for the following reasons: (*a*), the composition of the bile varies in different animals; (*b*), in the guinea pig variable amounts of bile may be lost when the cystic duct is cannulated and must be replaced with some sort of physiological saline solution; (*c*), and it is possible that the bile salts may, by contact with the mucosa or by diffusing through the mucosa, affect the excitability of the musculature.

Experiments were performed on the gall bladder of eight guinea pigs. In four the bile was not removed, and the response to the standard dose of CCK was determined. Then, the bile was removed and the viscus washed and then filled with S-R solution. The response to CCK was again determined. The reverse procedure was then performed on the other gall bladders.

As was observed by Jung and Greengard (6), no significant difference in response was observed whether the viscus contained bile or S-R solution.

However, it was found that the addition of 1.0 mgm. of desoxycholic acid to the bath caused the gall bladder to relax and decreased the response to CCK. Two milligrams of cholic acid caused no relaxation, but decreased the response to CCK. This confirmed previous observations that the intravenous injection of sodium glycocholate in the dog caused the gall bladder to relax (8). These observations showed that one should remove by washing with S-R solution any bile spilled on the gall bladder during cannulation of the cystic duct. They also indicated that after a gall bladder containing bile had remained in the bath for a number of hours, its musculature might lose irritability due to the diffusion of bile salts through the gall bladder mucosa. Regardless of this latter possibility we did not remove the bile from the gall bladder of the guinea pigs for the experiments to follow because we did not desire to disturb the original

volume-pressure relationship of the viscus, since we had found that washing the viscus before placing it in the bath irritates it.

The longitudinal shortening of the guinea pig's gall bladder in response to a standard dose of CCK at different intravesical pressures. The longitudinal shortening of the gall bladders of five guinea pigs to a standard dose of CCK was determined by the device shown in figure 1. The amount of shortening manifested by the gall bladders at different intravesical pressures is illustrated by the composite graph shown in figure 2.

The data from which curve *A* was constructed were obtained by recording the response to the standard dose of CCK with the viscus manifesting its original pressure-volume relationship. Then, the intravesical pressure was decreased at intervals to 1 cm., the response to CCK being obtained at each pressure level. After this the pressure was increased at intervals, the response to CCK being obtained at each new pressure level, until no or a very slight shortening resulted. Then, the pressures were

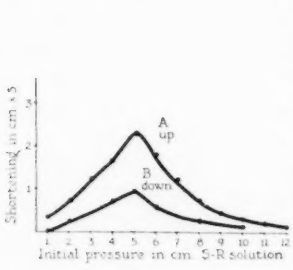

Fig. 2

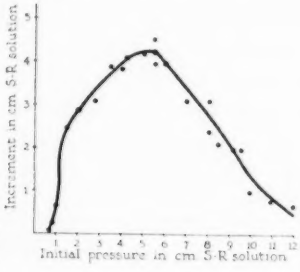

Fig. 3

decreased. The data from which curve *B*, figure 2, was constructed were obtained from two of the five gall bladders in which on reducing the pressure, the values of curve *A* were not reobtained. Evidently these gall bladders had been "over-stretched," or some factor had modified these gall bladders so that their responsiveness to CCK or their contractility was decreased. These gall bladders were considerably distended with fluid even when the pressure was reduced to 1 cm., indicating that "permanent" stretching had resulted.

It should be added that at the higher pressures the longitudinal muscle might relax in response to the addition of CCK before it contracted. Also, in the instance of several gall bladders after the pressure was decreased by decreasing the volume of fluid in the gall bladder, the musculature of the vesical contracted so firmly that it could be distended only by abnormally increasing the pressure. The results on such gall bladders were discarded; they would at times remain "spastic" for two hours; they apparently would not adapt or return to their original pressure-volume state.

The increase in intravesical pressure (guinea pig) in response to a standard dose of CCK at different initial intravesical pressures. These experiments were performed with the gall bladder suspended from the cannula and not attached to the muscle lever; thus, the intravesical pressure rise, in response to CCK, resulting from the contraction of both the circular and longitudinal musculature was obtained. One might wonder why we did not record the shortening of the gall bladder and the rise in intravesical pressure synchronously. We did in four experiments, and found no strict correlation between the two, because the pull or friction of our muscle lever apparently "distorted" or interfered with the contraction of the circular muscle.

In figure 3 is graphed the data obtained from the gall bladder on which it was possible to obtain the largest number of determinations before the irritability or contractility of the viscus manifested a definite change. In figure 4 is graphed (curve *A*) the data obtained on eight gall bladders.

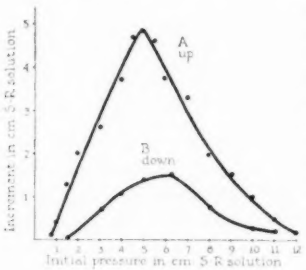

Fig. 4

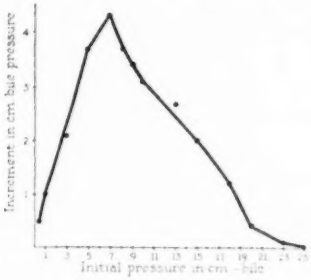

Fig. 5

(A minimum of ten determinations was obtained on each of eight gall bladders.)

Three of the eight gall bladders manifested a definite decrease in responsiveness to CCK after the intravesical tension had been raised to 12 cm. The data obtained from these three gall bladders, as the pressure was reduced after raising the pressure to 12 cm., are graphed as curve *B*, figure 4. These three gall bladders were "atonic" or "overstretched," since when the intravesical pressure was decreased to 2 cm. they contained more fluid than they held at that pressure at the start of the experiment. Again in this group of experiments two gall bladders became "spastic" when their volume was decreased. Five of the eight gall bladders from which the data in curve *A*, figure 4, were obtained, showed approximately the same volume at pressures of 4.5 to 5.5 cm. at least thrice during the periods of changes in initial pressure above and below those pressures. This indicates that these five gall bladders tolerated the experimental conditions well, and did not become spastic or atonic for a period of six hours.

While performing this and the former group of experiments two gall bladders markedly distended with bile were attached to the apparatus and placed in the bath. They failed to respond at their initial pressure to the usual 0.1 mgm. dose of CCK. They, however, responded to a 0.5 mgm. and 1.0 mgm. dose respectively. After responding to these doses the former responded to 0.1 mgm. at 4, 3, and 1 cm. of initial pressure

TABLE 1

Some of the data showing the relation between intragall-bladder pressure level, optimal pressure for optimal amplitude of tonus rhythm, and the optimal pressure for an optimal contraction

G. P. 1:									
A. Gall-bladder pressure in mm.	8	30	40	50	70	90	100		
B. Amplitude of tonus rhythm in mm.	0	2	1–2	2–6	3	0	0		
C. Pressure increment due to contraction in mm.	5	31	32	77	48	21	11		
G. P. 3:									
A	10	20	40	50	55	60	90	105	120
B	0.5	1	1–2	3–8	3–8	3–5	2–3	1–2	0.5
C	7	28	38	44	40	40	21	10	8
G. P. 4:									
A	10	15	20	35	45	55	75	105	115
B	0.5	1	2–4	3–8	3–8	1–2	1	0.5	0.5
C	2	8	40	57	40	26	19	7	5
G. P. 7:									
A	10	20	30	40	50	60	70	80	90
B	1	2		10	10	4	2	1	0
C	7	30	38	49	62	53	30	13	3

TABLE 1A

Guinea pig number	1	3	4	7	9	10	15	16	17	19
Pressure for optimal amplitude of tonus in mm.	50	50 55	35 45	40 50	40	60	50	60	50	60
Pressure for optimal contraction in mm.	50	50	35	50	40	60	50	60	50	60

and then became spastic, and the latter responded similarly at several pressure levels and then became atonic.

It is clear from the data obtained from the isolated guinea pig's gall bladder that the optimum increment of pressure resulting from a standard dose of CCK (0.1 mgm. of the typical case) occurs when the initial intravesical pressure is in the range of from 4.5 to 5.5 cm. This was the initial pressure at which the maximum tonus rhythm was observed. The excur-

sion of the tonus rhythm occasionally amounted to from 0.5 to 1.0 cm. pressure. When the pressure in the gall bladder was raised or lowered, the tonus rhythm would decrease. Further, when the tonus rhythm was maximum, the gall bladder responded almost immediately to CCK. At a pressure of 1 cm. or 10 to 12 cm., the gall bladder would not contract at times until after a latent period of five minutes. At the high pressures, the viscus might relax slightly before it contracted.

The maximum capacity of the isolated gall bladder of the guinea pig to contract in response to the standard dose of CCK was 12.5 cm. It was found in other experiments, however, that when the gall bladder failed to respond to the standard dose (0.1 mgm.) of CCK because of a high initial pressure (12.5–14.0 cm.), the addition of 0.5 mgm. of CCK would cause a contraction. The maximum pressure exerted under such conditions was found to be 14.8 cm. That is, we found no guinea pig's gall bladder which in response to CCK would support a column of S-R solution to a greater height than 14.8 cm.

The response of the dog's gall bladder in situ at different pressure levels. The observations on the isolated gall bladder of the guinea pig were repeated on the gall bladder in situ of the dog, 0.5 mgm. of CCK being used as a submaximal dose. Ten dogs were used. The results are illustrated by the composite curve in figure 5.

It is to be noted that the optimal initial pressure for the maximal increment of pressure in response to the standard dose of CCK ranged from 5 to 8 cm. for the different gall bladders. This range is somewhat higher than that observed for the isolated guinea pig's gall bladder. Also, as might be expected, the capacity of the dog's gall bladder to contract against pressure is twice that of the isolated guinea pig's gall bladder. Other observations of interest were made and will be briefly mentioned. At a pressure of 23 cm. of bile only two of the gall bladders responded to the standard dose. At a pressure of 20 cm. three, and at a pressure of 25 cm. one of the gall bladders responded to twice the standard dose of CCK. One gall bladder was found which contracted (1.4 cm.) in response to 1 mgm. of CCK (4 dog units) when the pressure was 35 cm. of bile. It should be mentioned that these latter experiments involving the maximal capacity of the gall bladder to contract were performed terminally or after all the determinations with the standard dose of CCK had been made.

The effect of prolonged distention of the gall bladder on its response to cholecystokinin. Only one gall bladder responded differently (diminished contractility) after the pressure had been raised to 22 cm. and then decreased to the initial pressure. But, since in life it is possible that the gall bladder may be subjected to a pressure of 22 cm. of bile for several hours, the following experiment was performed. The response of the gall bladder, at its initial pressure found after cannulation, to 0.5 mgm. of CCK

was determined, and then the pressure was raised to 22 cm. of bile by introducing gall-bladder bile (38°C.) from another dog. The pressure was kept at this level for two hours, and then reduced to the initial pressure. After the pressure and volume became adjusted at the initial level, 0.5 mgm. of CCK was again injected and the response measured. After the gall bladder relaxed the pressure was raised to 30 cm. and maintained there for 2 hours; then the pressure was reduced to the initial level. After the pressure and volume was adjusted at the initial level, 0.5 mgm. of CCK was injected and the response measured. The same procedures were repeated

TABLE 2

DOG NUMBER	INITIAL PRESSURE IN CM. OF BILE	RESPONSE TO CCK BEFORE PRESSURE CHANGE	RESPONSE TO CCK AFTER 22 CM. PRESSURE FOR 2 HOURS	RESPONSE TO CCK AFTER 30 CM. PRESSURE FOR 2 HOURS	RESPONSE TO CCK AFTER 40 CM. PRESSURE FOR 2 HOURS
	cm.	*cm.*	*cm.*	*cm.*	*cm.*
3	2.1	1.0	1.3	0.4	
10	5.4	2.0	1.9	2.0	
2*	5.8	1.0	1.6	2.5	
7	6.3	1.0	0.6	0.6	
12	6.4	2.8	1.8		1.0
6	6.5	2.0	1.8	1.6	
15	7.5	2.3	2.0		0.8
14	8.4	1.6	1.5		1.0
4	9.2	0.7	0.5	0.4	
8	10.0	1.0	0.5	0.2	
9	10.8	1.0	1.2	0.8	
13	11.2	2.0	1.3		0.6
1	12.0	2.0	1.8	2.6	
11	13.1	3.1	2.2		1.1
5	19.4	1.4	0.7	0.6	
Average.....	8.9	1.66	1.4	1.2	0.9

* Accidentally spilled bile in cannulation and 15.7 cc. of bile were required to raise pressure from 5.8 to 22 cm. of bile pressure. The data probably should not be included.

before and after maintaining the pressure in the gall bladder at 40 cm. of bile for 2 hours. The average amount of bile required to raise the pressure in the gall bladder from the initial pressure to a pressure of 22 cm. was 5.1 cc., and the amount removed to restore the initial pressure was 4.7 cc. The average amount required to raise the pressure from the initial to 30 cm. was 6.95 cc., and the amount removed to restore the initial was 6.7 cc. The values for a pressure of 40 cm. were 8.2 cc. to raise and 6.6 cc. to return.

The results are recorded in table 2. It is to be noted that maintaining the pressure in the gall bladder at 22 cm. of bile for 2 hours had an insig-

nificant effect on the response to CCK. The response was diminished but not markedly by 30 cm., but very definitely by 40 cm. The dog's gall bladder in situ withstands pressure remarkably well in comparison to the isolated gall bladder of the guinea pig.

Regarding the tonus rhythm of the gall bladder in the dog. The tonus rhythm of the dog's gall bladder in situ is subject to too much individual variation, and is affected too much and for too long a period by a preceding injection of cholecystokinin to render possible the tabulation of data in the manner that is possible for the isolated organ of the guinea pig.

Only about one-half of the gall bladders manifested an appreciable tonus rhythm at the initial pressure prior to the injection of CCK, but they all showed a tonus rhythm after the first injection of cholecystokinin, the amplitude of which varied from 3 mm. to 20 mm. The tonus rhythm initiated after the first injection frequently continued throughout the 6 or 10 hours of the experiment. The tonus rhythm was present frequently at 0 pressure; and, even at 20 or 25 cm. of pressure, it could be present when the viscus failed to respond to the standard dose of CCK. For example, one gall bladder showed a rhythm having an amplitude of 1 cm. at a pressure of 25 cm., and at which it did not respond to the standard dose of CCK. Yet, the amplitude was clearly influenced by the resting or pre-injection intravesical pressure, it being lowest at zero and at 20 to 25 cm. of bile pressure. The range of pressure for the optimum amplitude was wide, namely, from 3 cm. to 15 cm. The most typical change in the amplitude of the rhythm was that it was definitely diminished or frequently almost disappeared at the height of the contraction caused by CCK (7). These results confirm our previous experience in regard to the observation that cholecystokinin not only causes a contraction of the gall bladder as a whole, but also causes a tonus rhythm to appear or, if present, causes an increase in its amplitude and frequency (7), except during the height of the contraction. The results also show that the range of optimal tonus rhythm is much wider for the dog's gall bladder than for the guinea pig's and that less correlation exists between the range for optimal amplitude of the rhythm and the range for optimal contraction in response to CCK in the dog than the guinea pig. However, as in the guinea pig, the gall bladder responds more quickly to CCK when the tension is in the range for optimal contraction.

Discussion. Our observations show that the smooth muscle of the gall bladder manifests an optimal tension at which maximal contractions occur in response to a stimulus; or, the initial tension in the gall bladder determines its response to a standard submaximal dose of cholecystokinin. Our curves demonstrating this, conform in general with those obtained for the longitudinal muscle of the cat's ileum by Brocklehurst (1) and the nictitating membrane of the cat by Hampel (2), although there are differ-

ences in detail. Because of the anatomy of the gall bladder when studied as a whole, it is more difficult to set up ideal experiments than in the case of the nictitating membrane, the intestine or uterus (3). We believe any differences in detail are to be explained on the basis of the technical factors and the type of contraction we were studying than on the basis of differences in fundamental properties of the smooth muscle of different organs.

In the case of the isolated guinea pig's gall bladder the optimum tension at which maximal contractions occur is in the range of tension at which the viscus manifests a tonus rhythm of greatest amplitude, and at which the musculature responds most quickly to CCK. An optimal tension for optimal tonus rhythm exists and, when the tension is increased or decreased by a change in volume, the tonus rhythm is diminished; or when the viscus is caused to contract, the tonus rhythm is diminished at the height of the contraction to return again during relaxation; or, if at a low pressure (2 cm.), and if a rhythm of low amplitude is present and the viscus is caused to contract, a tonus rhythm of large amplitude generally follows when the contraction raises the tension to the range optimal for a tonus rhythm. This is contrary to the observations of Simeone (3), on the cat's uterus, which indicated that an increase in tension diminished the amplitude and had no effect on the frequency of the tonus rhythm. Evans (9) finds that stretching renders smooth muscle more excitable and institutes a tonus rhythm and that the greater the stretch the greater the frequency. Ritchie (10) interprets the work of Brown and McSwiney (4) as indicating that preparations of stomach showing the most tone show the least rhythm and vice versa, and supports the view that stretch is the stimulus responsible for "tone." We find in the case of the gall bladder that excessive tension diminishes the amplitude of the rhythm, as in the case of the uterus (3), and may abolish it entirely. Simeone (3) believes that this fact is an argument against the view that "tone" is an effect of stretch. Yet, may not "tone" as a physiologic phenomenon exist independently of rhythmicity? Our experience with the gall bladder indicates that the discordant statements regarding the effect of tension on the amplitude of the tonus rhythm are only partially correct, and that the effect observed is related to the initial tension present when some stimulus is introduced, and depends in part on the nature of the stimulus. *We should conclude that an optimal tone or tension is generally requisite for a tonus rhythm of optimal amplitude, as well as for optimal excitability, and that this optimal tension may vary somewhat in the case of the gall bladder of the same and different species.* In the case of the dog's gall bladder in situ, CCK can augment the amplitude and also the frequency of the tonus rhythm without a change in initial basal "resting" tension, or with a sudden rise in tension (rapid contraction), the amplitude may be markedly diminished; or with a slow rise in tension (slow contraction), the amplitude

may be unaltered. These facts, however, do not negate the general truth of the preceding statement, because of the factor of optimal range which is subject to some variation.

The observations made during this investigation have several applications regarding the mechanism of gall-bladder evacuation. If the pressure in the gall bladder is low, more CCK will be required to cause it to contract than if its pressure is in the range of 5 to 15 cm. of bile (dog). If the pressure is high (20–25 cm. of bile), a larger quantity of CCK will be required to cause contraction than when its pressure is somewhat lower. Or, when the pressure is high, the initial evacuation would be more dependent on a relaxation of the "sphincter of Oddi," leading to a passive flow of bile into the intestine, than on excitation of the gall bladder by CCK. After the initially high pressure had been reduced somewhat, then CCK would be effective in causing an active flow and in maintaining the pressure in the gall bladder at a level adequate for further evacuation. (It should be recalled that only one dog was found in which the gall bladder manifested some contraction at a pressure of 22 cm. when a relatively large dose of CCK was given.) Further, if the pressure in the gall bladder has been excessively high for a period, this may lead to a more or less "permanent" stretching or atony (curve *B*, fig. 4) of the viscus, so that a relatively large concentration of CCK in the blood would be required to cause the viscus to contract, and then it would not evacuate as completely as a "normal" gall bladder.

Roentgenologists have frequently asked one of us (A. C. I.) the following question: Given a patient whose stomach empties egg yolks and cream well and whose gall bladder does not empty, if the failure to empty is due to a hypertonic sphincter, why does not the patient suffer biliary tract distress always? The reply has been that possibly CCK was not produced in an adequate amount to cause a forceful contraction. Two other possible answers are now provided. One, the gall bladder of the patient may have been "overstretched" so that until the viscus recovers from the "overstretched" or atonic state, CCK must be present in unusual amounts to cause a rise in intrabiliary tract pressure adequate to cause distress. Second, if the pressure in the patient's gall bladder is high (20 cm.), an unusually large amount of CCK must be present to raise the pressure suddenly to the point where distress is produced.

SUMMARY AND CONCLUSIONS

1. The tension developed by the gall bladder when it contracts in response to a single submaximal dose of cholecystokinin depends on the initial pressure.

2. The optimal intragall-bladder pressure for an optimal contraction in response to a submaximal dose of the hormone was found to vary from

4 to 5.5 cm. of pressure (Sollmann-Rademaeker's solution) in the isolated vesicle of the guinea pig and from 5 to 15 cm. of bile pressure in the dog's vesicle in situ. When the pressure is optimal the vesical contracts more quickly than otherwise. The guinea pig's gall bladder failed to contract in response to the standard dose of the hormone when intravesical pressure was 12.5 cm.; this value for the dog was 25 cm. At these high pressures a slight contraction might be obtained when the standard submaximal dose of the hormone was increased from two to four times. If the gall bladder is "overstretched" by the relatively high pressure, then the viscus contracts less in response to the hormone.

3. The range of optimal pressure for an optimal contraction is approximately the same as the optimal pressure for a tonus rhythm of optimal amplitude. This was found to be true of the isolated gall bladder of the guinea pig.

4. If the gall bladder of the dog is subjected to a pressure of 30 or 40 cm. of bile for two hours, it contracts less to the same dose of cholecystokinin than it did before.

5. These observations must be considered in the interpretation of the response of the gall bladder to a meal.

We desire to thank Messrs. M. M. Pomarane and A. L. Berman for assisting us in certain phases of our work.

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THE EFFECT OF ETHER ANESTHESIA ON THE VOLUME OF PLASMA AND EXTRACELLULAR FLUID[1, 2]

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The extensive clinical and experimental use of ether demands a thorough understanding of the physiological action of this anesthetic. The purpose of the experiments reported here was to determine what effects, if any, ether has on the volume of plasma and of extracellular fluid.

There are two lines of evidence which indicate that ether anesthesia reduces the plasma volume. One is that ether causes hemoconcentration (Epstein, 1917; Barbour and Bourne, 1923, and others), and the other that less than the estimated normal quantity of blood is recovered by gross exsanguination from etherized dogs (Mann, 1916). It should be pointed out, however, that hemoconcentration is evidence of fluid loss only if it be assumed that the total amount of circulating cells or solids remains unchanged during the experiment. That this assumption may be untenable has been shown by Searles and Essex (1936) who found in dogs that the rise in hematocrit reading caused by ether was reduced one-half after splenectomy. Exsanguination studies are also inconclusive, first because it is impossible to make control measurements on the same animal, and second, because it is difficult to effect complete exsanguination

Some indication of the behavior of the extracellular fluid in dogs during ether anesthesia is furnished by the electrolyte studies of Austin, Cullen, Gram and Robinson (1924). In a representative experiment these workers found that ether caused a decrease of 4 Mm/L in the concentration of blood total base. If it be assumed, as Gamble (1937) has proposed, that the majority of the basic ions maintain an exclusively extracellular position, this decrease represents an increase of approximately 2.5 per cent in the volume of extracellular fluid, a scarcely measurable amount. In the experiments described below an attempt has been made to find evidence of a change by measuring the fluid available for the solution of thiocyanate before and during etherization.

A preliminary report of this work was presented at the meeting of the American Physiological Society, Memphis, 1937.

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METHODS. The experiments were performed on dogs. The animals were given water ad libitum, but no food for 16–20 hours before the experiments. They were placed on the dog board and kept quiet for about

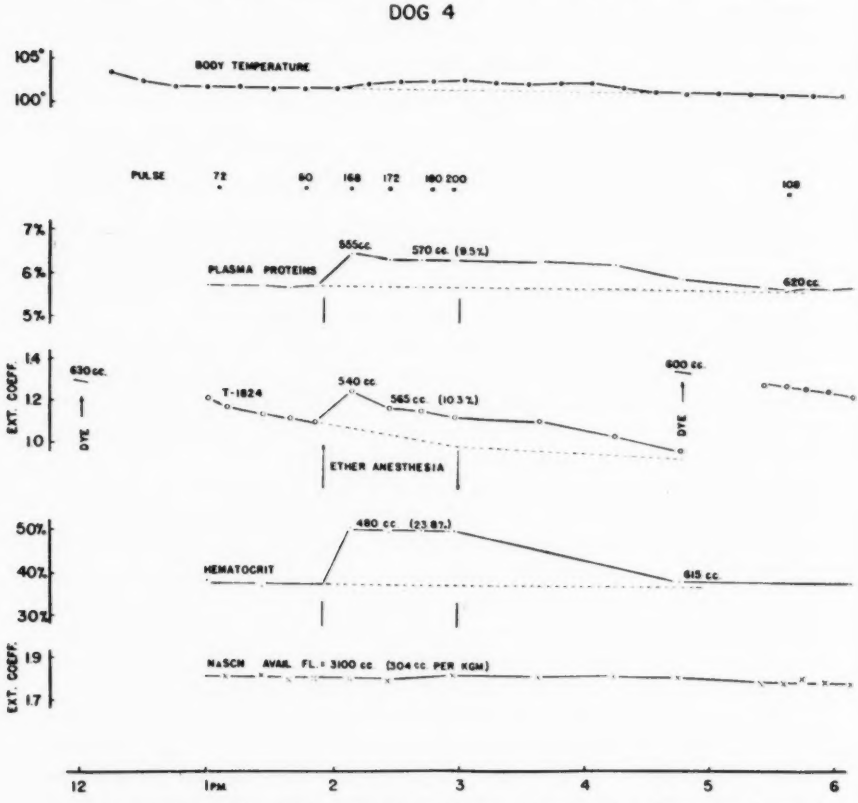

Fig. 1. April 2, 1937. Male dog, 4M, 10.2 kgm. Showing the effect of one hour of ether anesthesia on body temperature, heart rate, plasma protein concentration, plasma volume as revealed by the serum concentration of the dye T-1824, hematocrit, and "available fluid" as revealed by the serum level of thiocyanate. The deviation in serum dye concentration from the projected disappearance curve, accompanied by a similar deviation in the level of the plasma proteins and the hematocrit indicates a loss of fluid from the circulating blood. Room temperature before anesthesia, 76.4°F–79°F; during anesthesia, 77.8°F–78.2°F; after anesthesia, 76.5–80.8°F.

two hours prior to the administration of ether by the drop method. The initial ether apnea was eliminated by having an assistant compress the thorax to force respiration. Complete narcosis was thus induced within from one to two minutes, and struggling was reduced to a minimum.

Anesthesia was maintained for one hour at such a depth that the conjunctival reflex was just present and the tongue was of good color. Blood samples were drawn without stasis from the jugular veins, and rectal temperature, pulse and respiration were recorded at frequent intervals.

The plasma volume was measured with the blue dye T-1824, determined spectrophotometrically in serum samples (Gregersen et al., 1935, 1937, 1938). A known amount (usually 2 cc.) of one per cent dye solution was injected about 2 hours prior to etherization. The initial plasma volume was calculated from the dye-concentration obtained by projecting the control disappearance curve back to the time of injection (see fig. 1), thus correcting for the dye lost from the bloodstream during the period of mixing. Changes in the plasma volume which occurred during and after anesthesia were estimated from the deviations in the time-concentration curve of the dye from its original course. Allowance was made for the fact that the disappearance rate is normally somewhat less 3 to 4 hours after the injection than during the first 2 or 3 hours. Dye was again injected at the end of most of the experiments and the plasma volume at that time calculated from a new disappearance curve. Changes in plasma volume were also estimated from the variations in the serum protein concentration (measured with a refractometer, Neuhausen and Rioch, 1923) and from hematocrit readings (made with Wintrobe tubes and powdered heparin). The method of plotting the results is illustrated in figure 1.

The volume of fluid available for the solution of thiocyanate was determined according to the directions of Gregersen and Stewart (1938), who have demonstrated that this procedure may be carried out simultaneously with measurements of plasma volume with T-1824. In this technic the spectrophotometer is used for analyzing the final color reaction of thiocyanate. The results have been expressed simply as "available fluid," no attempt being made to correct for thiocyanate in the erythrocytes or the possible unequal distribution of thiocyanate between plasma and extravascular fluid (Crandall and Anderson, 1934; Lavietes et al., 1936). While the values do not represent exactly the volume of extracellular fluid, it is felt that changes in the serum concentration of thiocyanate should reflect changes in the extracellular fluid volume and the order of magnitude of these changes.

Results. In 23 experiments, ether anesthesia for one hour caused an average reduction of 11.0 per cent in the plasma volume. Table 1 shows that there was considerable variation in the amount of fluid lost, ranging from 6.3 to 17.2 per cent. It should be noted, however, that repetition of the experiments on dogs 1M and 2M gave practically the same result each time. Although a few experiments showed a continuous decrease of plasma volume, the reduction was generally maximal within a few minutes after the induction of narcosis (see fig. 1). Within four

hours after the removal of ether, the volume of plasma had returned to the preanesthetic level and, in many experiments, it had measurably increased.

When estimated from the rise in concentration of the serum proteins, the decrease in plasma volume averaged 9.3 per cent. In some instances,

TABLE 1

The effect of ether anesthesia on the volume of plasma and "available fluid"

DOG NUMBER	DATE	WEIGHT	PLASMA VOLUME		PER CENT REDUCTION OF PLASMA VOLUME CALCULATED FROM				"AVAILABLE FLUID"		CHANGE IN VOLUME OF AVAILABLE FLUID
					Dye	Plasma protein	Hematocrit				
							Normal	Splenectomized			
		kgm.	*cc.*	*cc./kgm.*					*cc.*	*cc./kgm.*	*per cent*
1M	11/23/36	12.5	825	66.0	14.0	6.0			4,370	350	0.0
	12/ 9/36	12.1	730	60.5	13.7	11.0					
	1/ 8/37	12.5	740	59.5	14.9	10.8	19.6		4,165	335	0.0
2M	12/10/36	14.9	895	60.0	10.0	8.4	8.4				
	1/ 4/37	15.0	915	59.4	10.4	12.0			4,900	318	0.0
	1/26/37	16.4	930	57.0	9.7	8.1	17.2		5,320	326	0.0
3M	10/16/36	12.0	785	65.0	17.2	12.9					
	11/13/36	12.9	795	61.6	13.8	8.2			3,800	295	0.0
	7/13/37	12.8	715	56.0	9.1	7.0		5.6	3,670	286	−8.2
4M	1/ 5/37	10.8	605	56.0	8.3	8.3	13.6		3,070	284	0.0
	4/ 2/37	10.2	630	61.9	10.3	9.5	23.8		3,100	304	0.0
5M	12/ 3/36	13.6	715	52.5	11.2	7.7			4,200	324	0.0
6M	6/11/35	19.1	960	50.4	10.9	12.0					
7M	6/11/35	21.0	925	44.0	10.2	7.0					
8M	4/25/35	22.0	985	45.0	10.7	11.2					
9M	12/15/36	12.7	730	57.7	8.9	9.6					
10M	6/13/35	21.1	1060	50.0	14.2	7.5					
G	7/22/37	19.0	1090	57.5	6.5	9.2		7.4	6,560	350	0.0
22M	6/19/37	7.4	445	60.5	11.1	10.1	16.9		2,390	326	Irregular
23M	6/24/37	18.4	820	44.6	14.6	14.0	23.2		5,210	283	−3.3
24M	5/14/37	12.0	595	49.6	8.4	9.3	21.9		3,510	293	+8.0
25M	1/ 3/36		545		11.0						
26M	5/ 8/37	9.3	560	60.3	6.3	4.5	19.6		2,940	316	Irregular
Average				56.1	11.0	9.3	18.2			314	0.0

there was a considerable difference between the value calculated from the increase in the serum proteins and that calculated from the rise in the concentration of the dye. Since the discrepancy is generally in the direction of lower protein values, it is conceivable that some of the smaller protein fractions leave the circulation along with the fluid. It should

be noted, however, that refractometric measurements of the serum protein concentration are not absolute.

The average loss of plasma estimated from the increase in the hematocrit was 18.2 per cent, almost double that calculated by the other methods. In two splenectomized dogs, the rise in the hematocrit was less pronounced, thus furnishing indirect evidence of the splenic contraction during etherization which has been observed by Barcroft (1929), Bhatia and Burn (1933) and Hausner, Essex and Mann (1938).

The plasma volume reduction was independent of the fall in body temperature usually caused by ether. It may be seen that whereas the reduction of plasma volume occurred in all experiments, in four the rectal temperature remained constant throughout anesthesia, and, in two experiments, it rose as much as 1°F. In the latter, the rise in temperature may have been due to the high room temperatures (75°–90°F.) prevailing at the time. Barbour and Bourne (1923) found that body temperature during ether anesthesia was best maintained at a room temperature of 88°F. The present studies showed that it may take as long as one and one-half hours for the body temperature of resting dogs to reach a basal level. Hence, the absence of the customary fall in body temperature in some of these experiments may also have resulted from the establishment of a true basal temperature prior to the anesthesia.

The average heart rate of the dogs at rest and before etherization was 90 beats per minute, whereas after one hour under anesthesia it was 173 beats per minute.

The effect of ether on the volume of "available fluid" was investigated in 14 experiments. The average of all the results indicates that there was no measurable change, but it should be stressed that this is not invariably the case. For instance, in one experiment there was a decrease of 3.3 per cent, in another a decrease of 8.2 per cent, in a third an increase of 8.0 per cent and in two experiments the results were irregular. The remaining 9 showed no measurable change in the "available fluid."[3]

Discussion. The results of these experiments confirm the general belief that ether anesthesia causes a reduction of plasma volume in dogs. The method employed furnishes a quantitative measurement of plasma volume in the same animal both prior to and during the anesthesia. The measuring agent, moreover, is a foreign substance which, unlike blood cells or plasma solids, is not affected by endogenous metabolism or the presence of depots.

Inasmuch as the injected dye is initially confined within the vascular system, as evidenced by the slow disappearance rate, variations in the

[3] Stewart and Rourke (1938) have reported recently that patients subjected to surgical operation under ether show a marked increase in "available fluid" volume.

disappearance curve can be caused only by a change in the rate of excretion of the dye, and/or by an exchange of fluid between the circulatory and extravascular compartments. Dyes of the group to which T-1824 belongs are excreted largely in the bile (Smith, 1925, 1930), and, since ether is believed to interfere with liver function (Rosenthal and Bourne, 1928), it might be thought that the rise in dye concentration during etherization could be accounted for by a diminished rate of excretion. Reduced excretion of itself could not, however, cause the dye concentration to rise above that obtained at an earlier period (see fig. 1). Furthermore, the simultaneous and proportionate increase of serum protein concentration, and of red cell concentration in the splenectomized dogs shows quite clearly that the observed effect is one of fluid exchange rather than disturbed liver function.

The mechanisms underlying this fluid exchange have not yet been determined. Mann (1916) suggested that the low blood volume during ether anesthesia was caused by an extravasation of fluid resulting from venous stagnation and loss of muscle tone. A different view was taken by Epstein (1917), who thought that the hemoconcentration he observed was occasioned by the ether acidosis which caused the hydrophilic tissues to absorb fluid from the blood. The calculations based on previous electrolyte studies and the lack of significant or consistent changes in "available fluid" volume during etherization point strongly against such a view of fluid absorption by tissue cells. Both investigators assume that there is a direct extravasation of plasma into intercellular spaces or into the tissues themselves, but it should be noted that the circulating fluid may also be depleted by loss through the kidneys, lungs and various secretory organs. Actually, the kidneys would tend to produce the opposite effect, for renal output may become so low during ether anesthesia that complete anuria results (Hawk, 1911). According to Barbour and Bourne (1923) water loss through the lungs is not excessive under ether. Of the various secretory organs, the salivary glands demand consideration because of the profuse flow of saliva which ether excites. The impression gathered during the experiments, however, was that the salivary flow was not sufficient to account for the plasma loss. Hence, the plasma volume does not seem to be depleted by the excessive removal of fluid through kidneys, lungs or secretory organs, but rather by a movement of fluid into the interstitial compartment as a result either of mechanical alterations in the circulation or of general chemical changes or both.

SUMMARY

1. The effect of ether anesthesia on the plasma volume of dogs has been measured with the blue dye T-1824 and spectrophotometric analysis of serum samples. In 23 experiments one hour of anesthesia caused an average decrease in plasma volume of 11.0 per cent.

2. When calculated from the rise in concentration of the serum proteins, the fluid loss averaged 9.3 per cent.

3. The hematocrit rise was almost double that which could be accounted for by the plasma volume reduction. This discrepancy was less pronounced after splenectomy.

4. Within four hours after the removal of ether, the plasma volume returned to the preanesthetic level and, in some cases, it rose well above that level.

5. The decrease of plasma volume was independent of changes in body temperature.

6. As indicated by measurements of the fluid available for the solution of sodium thiocyanate, there was no consistent change in the volume of extracellular fluid.

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POLY-LOBATION OF THE POLYMORPHONUCLEAR NEUTROPHILE AS A RESULT OF WORK AND TIME AGEING[1]

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For many years hematologists have been interested in the significance of the nuclear lobation in the granular leucocytes, especially in the case of the polymorphonuclear neutrophile. Arneth (1) originally suggested that lobation of the polymorphonuclear leucocytes could be used as an index of age. Some investigators believe that poly-lobation is the result of time alone, while others believe that it may result from increased activity on the part of the cell (Cooke, 2; Ponder, 3; Cooke and Ponder, 4; Climenko, 5; Climenko and Ponder, 6). It is difficult to obtain direct evidence showing that the cells change from class I to class II and from class II to class III, etc., as their age increases. Climenko and Ponder (6), nevertheless, offer data to indicate this occurs under experimental conditions in isolated veins. In the present work, it is believed that ageing effect can be shown to occur not only as an index of time but also as a result of experience.

MATERIALS AND METHODS. Materials studied consisted of sections through the uteri obtained from pregnant rats. In each case the uterus contained a fetus which had been killed on the fourteenth day of gestation and retained in utero for varying lengths of time (from 12 hours to 8 days). The curettage method of killing the fetus was employed (Crosman, 7). Animals were sacrificed at different intervals within the above time range, and the uterus containing the dead fetus was removed. The material was fixed, sectioned serially and stained with hematoxylin and eosin. This gave a series of cases which only differed from one another in gradually increased retention time. A region through the center of the placenta and umbilical cord was selected in each case in order that the areas utilized for the purposes of the polynuclear count would be as near comparable as possible. In the selected areas, counts were made of the neutrophiles found in the uterine blood vessels and in the uterine cavity. The latter counts were restricted to the areas immediately surrounding the autolyzing

[1] Presented at the Annual Meeting of the American Society of Anatomists at Pittsburgh, Pa., 1938.

placenta and fetus. Counts were also made from smears of the peripheral blood as a check on the condition of the colony and were found to give an average weighted mean of 1.15 for six counts. This compares favorably

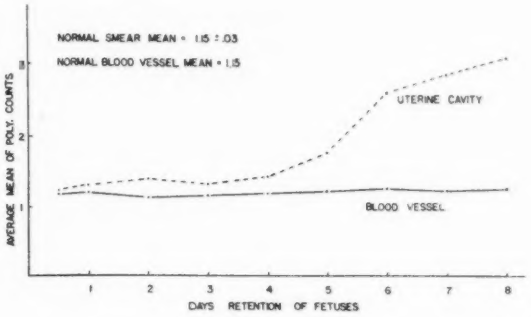

Fig. 1

TABLE 1

Polynuclear count on cells in uterine blood vessels and uterine cavity

CASE	RETAINED	*BLOOD VESSELS				CAVITY						WEIGHTED MEANS	
		I	II	III	IV	I	II	III	IV	V	VI	Bl. Ves.	Cavity
	days												
1	½	87	11	2	0	80	17	3	0	0	0	1.15	1.23
2	½	85	12	3	0	79	19	1	1	0	0	1.18	1.24
3	1	82	16	2	0	74	21	4	1	0	0	1.20	1.32
4	1	84	10	6	0	76	20	2	2	0	0	1.22	1.30
5	2	88	11	1	0	70	24	4	2	0	0	1.13	1.38
6	3	85	14	1	0	74	21	4	1	0	0	1.16	1.32
7	4	82	18	0	0	70	22	4	3	1	0	1.18	1.43
8	4	83	16	1	0	72	20	3	4	1	0	1.18	1.42
9	5	82	15	3	0	60	18	15	6	1	0	1.21	1.70
10	5	80	18	2	0	56	19	17	5	3	0	1.22	1.80
11	6	80	16	3	1	35	18	20	11	12	4	1.25	2.59
12	7	81	17	1	1	27	9	32	7	16	9	1.22	2.83
13	8	82	13	4	1	20	14	36	6	20	4	1.24	3.04
14	8	81	15	3	1	22	10	38	7	18	5	1.24	3.04

* No cells of class V or class VI were found.

with the mean of 1.16 obtained by Yeager and Haterius (8) working with the same colony.

RESULTS. An examination of table 1 and the graph shows that the polymorphonuclear neutrophiles in the blood vessels of the uterus of experimental animals exhibit a tendency to right deflection after twenty-

four hours retention. This is followed by a left swing after 48 hours and a shift to the right. After eight days' retention, the weighted mean is 1.24.

The cells in the uterine cavity show a steady right shift through 48 hours' retention, after which there appears a slight movement to the left, followed by a steady rise commencing at the 72 hour retention period, becoming more apparent following the 96 hour interval and is sharpest directly after the fifth day, to continue until, at the eighth day, the weighted mean of 3.04 is reached.

Discussion. Correlating the progressive autolytic changes occurring in the disintegrating fetus, as described by Crosman (7), with the present data it is interesting to note that apparent tissue changes are taking place in the retained material at the time when the deflections are noted in the counts. The changes in the retained embryos are gradual in the first three days. Starting with the fourth day, however, the variations in rate of autolysis in the different organs have become definitely noticeable. It is at this time that the largest numbers of leucocytes are present in the tissues of the uterine wall, whence they appear to be migrating in large numbers into the uterine cavity. They reach the greatest concentration in the uterine cavity at about the eighth day. This histological picture is reflected in the sharp rise of the weighted mean obtained in the present work, indicating an increase in the number of poly-lobated cells or in the lobation of cells already present. In either event, the cross section of leucocytic population presents evidence of rapidly ageing cells.

Considering time as the primary factor, Ponder (3) and Climenko and Ponder (6) showed an increased lobation of the nucleus of the polymorphonuclear neutrophile to occur directly with an increase in time. Their evidence indicates that a lapse of weeks is necessary to cause the leucocytes to shift from class I through the higher classes. This shift is usually referred to as time ageing. In the present work, it is quite evident that a similar shift requires not weeks but days, indicating that some accelerating factor other than time alone must be involved. Considering the conditions of the experiment, the other factor believed to be functioning is the increased activity on the part of the cells which seems to be necessary for them to cope with the foreign material in the form of the disintegrating placenta and dead fetus present within the cavity of the uterus.

CONCLUSIONS

1. Using increased nuclear lobation as a criterion, evidence is offered that the rate of ageing of the polymorphonuclear neutrophile is increased in the region of the retained autolyzing material in the uterus of the rat.

2. The region of autolysis shows increased concentration and activity of leucocytes. This increased activity functions as an ageing stimulus on the polymorphonuclear neutrophile, bringing about an increased loba-

tion in a shorter time limit than that required for a similar lobation with time alone as a factor.

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DEPRESSOR EFFECT OF INTACT RABBIT RED BLOOD CELLS IN THE DOG[1]

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In the course of an investigation of the effect of various constrictor and dilator drugs upon coronary blood flow in the perfused cat's heart (1), it was found that substitution of dog for cat blood as the perfusing medium produced a marked and lasting vasoconstriction, similar to that described by previous workers (2, 3). In view of the conflicting statements that have appeared in the literature attributing either vasodilator (4, 5, 6) or vasoconstrictor properties (7, 8) or both (3, 9, 10) to blood, we decided to investigate this subject in the intact animal in the hope that a clearer insight might be gained into the mechanisms involved.

METHOD. The experiments were performed on 43 dogs, 6 cats and 3 rabbits, anesthetized with intravenous or intraperitoneal nembutal (27 mgm. per kgm.). Mean carotid blood pressure tracings were obtained on a kymograph with a mercury manometer. In some experiments kidney and limb volume tracings were noted as well, using an oncometer and a hand plethysmograph respectively, with Brodie's bellows recorders. In other instances either the spleen alone or spleen, liver and intestines were removed *en masse*, according to the technique of Markowitz et al. (11). All injections were made into the femoral vein. The blood of the animals to be tested was prevented from clotting by the addition of either sodium citrate (0.1 cc. of a 30 per cent sol. per 10 cc. of blood) or heparin. The various constituents of the blood (thrice washed cells, plasma, serum, etc.) were obtained by the customary techniques.

RESULTS. I. Selection of test animal: *Cat.* In six experiments heterologous blood was injected into anesthetized cats. It was found that amounts up to 7 cc. of whole dog blood produced no effect upon blood pressure, while on the other hand, rabbit red blood cells ($\frac{1}{2}$–2 cc. of a saline suspension made up to original volume) consistently elicited definite depressor responses. Rabbit plasma acted similarly in 3 out of 5 experiments, no effect being produced in the remaining two instances. Ad-

[1] Aided by the Morris Loeb Fund.

ministration of whole cat blood obtained from other animals produced no effect.

Rabbit. The rabbit was used only three times because of the difficulty of maintaining a constant depth of anesthesia and the rapid formation of clots in the recording system. The injection of dog plasma (2–3 cc.) produced a fall in blood pressure, while the red blood cells of the dog and sheep had no effect.

Dog. The dog (preferably less than 12 kgm.) was used as a test animal in most of the experiments since its response to the injection of heterologous blood was found to be more marked than that in the cat and rabbit. The most effective depressor reaction in this animal was elicited by blood from the rabbit, followed in order of diminishing response by that from the rat, guinea pig, man, sheep and horse. The reactions with the latter bloods were neither constant nor predictable. Blood from cats or from other dogs was ineffective. Accordingly, the depressor phenomenon was studied using rabbit blood injected into the dog.

In an attempt to determine which constitutent or constituents of the blood were responsible for the vasodilator effect, the various portions obtained by centrifugalization were tested separately. It was found that both serum and plasma, even in dosages of 10 to 15 cc., were ineffective, while the injection of as small a quantity as 0.2 cc. of intact washed red blood cells (suspended in saline to original volume) produced the depressor effect. Removal of the white cells and platelets did not alter the response resulting from the injection of the remaining erythrocytes. On the other hand, washed red blood cells hemolyzed with either 3 volumes of distilled water or saponin elicited no effect. Special care had to be taken, however, to lake all cells, since the presence of even a few intact ones caused a depression of the blood pressure. Likewise, neither a fat nor a protein extract, made from packed red blood cells, produced a response. In other words, it appeared that the depressor effect was dependent upon the introduction of the intact red blood cell into the circulation of the dog, injection of the constituents of the cell itself (liberated by laking), as well as the other portions of the blood being totally ineffective.

II. Characteristics and type of response: *Time character of response.* It was consistently found that a definite latent period existed between the time of injection of the suspension of rabbit blood cells and the onset of the response. This lag generally ranged between 25 to 35 seconds, although at times it was as long as 60 to 65 seconds; in no instance was it less than 25 seconds. The entire response lasted from 2 to 3 minutes, after which the pressure regained its initial level (fig. 1-1C). This is in contrast to the results obtained with histamine, in which the drop in blood pressure began in about 8 to 10 seconds after administration and lasted

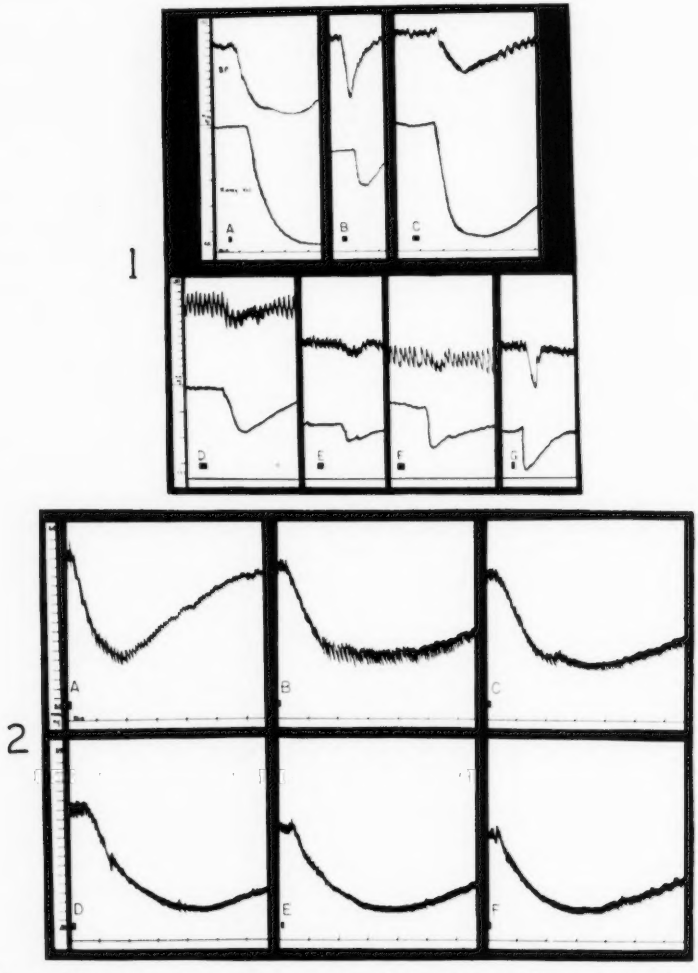

Fig. 1. Effect of repeated injections of rabbit red blood cells upon carotid blood pressure in 1, a normal dog, and 2, a splenectomized dog.

1. Normal dog (no. 14). Upper curve—carotid blood pressure. Lower curve—kidney volume. A, ½ cc. suspension of red blood cells. B, ½ cc. histamine, 1:10,000 dil. C, D and E, injections of ½ cc. suspension of red blood cells at 10 minute intervals. F, 2 cc. suspension of red blood cells. G, ½ cc. histamine, 1:10,000 dil.

2. Splenectomized dog (no. 22). Each record represents the effect of ½ cc. suspension of red blood cells injected at 10 minute intervals.

Compare graphs 1 and 2 as to magnitude of response with successive injections.

about one and one-half minutes. As a rule the initial injection of ½ cc. of washed cells produced a drop of at least 100 mm. Hg.

Refractory state. Generally it was found that repeated injections of the same dose of red blood cells (½ cc. of the suspension) at short intervals (10 min.) produced successively decreasing responses (fig. 1-1), until eventually only a slight effect was elicited even by four times the usual dose (2 cc.) (fig. 1-1F). These findings are in accord with those previously reported by Katz and his associates (2). The number of injections necessary to produce this "refractory" state varied in different experiments from five to twenty. In the majority of instances, an interval of more than an hour between injections permitted the "refractory" state to disappear, the first injection following this period being again effective in eliciting a definite drop in pressure.

In some experiments no gradation in response was observed with the first 2 or 3 injections; at times in fact the second administration produced a greater effect than the first. On the other hand, in a number of instances, the first response was very marked (a drop of as much as 130 mm. Hg) while the second was considerably reduced (a drop of 40 mm.). In these latter experiments, the animal generally became unresponsive after only a few injections.

Modification of "refractory" state by removal of spleen. When the spleen alone was removed at the beginning of the experiment, before the injections were begun, it was found that the phenomenon of the "refractory" state either no longer developed or was definitely modified. In many instances, the successive injections in the splenectomized animal produced almost equal depressor responses (fig. 1–2). Furthermore, in contrast to the intact animal, the time required for the blood pressure to return to the control level gradually increased during the experiment. These characteristic effects of splenectomy failed to appear, however, if the organ was removed after a number of injections of red blood cells had been previously made. Under these circumstances, the successive responses were similar to those observed in the intact animal.

III. Site of action: 1. *Removal of viscera.* In six experiments, after previous injections of rabbit red blood cells and histamine had demonstrated the responsiveness of the animal, the spleen, pancreas and intestines were removed and the injections repeated. In five instances, the response to red blood cells was ⅓ or less of the magnitude of the control (fig. 2); in one the decrease in the depressor reaction was less conspicuous. Subsequent removal of the liver in one of the former cases did not in any way change the response (fig. 2-E). Injection of six times the usual dose of suspended red blood cells into this animal increased the extent of the drop in blood pressure (fig. 2-F), indicating the absence of a "refractory" state. The fact that a depressor effect was still elicited after removal of

the splanchnic bed suggests that blood vessels in other regions also participate in the dilatation, but probably not to the same extent.

2. *Effect upon blood flow in an extremity.* Volume changes of the limb were recorded with a plethysmograph in three experiments, and in each instance a decrease in volume was observed; indicating that the dilator effect on these vessels, if present, was less than the passive changes resulting from the drop in general blood pressure.

3. *Effect upon kidney volume.* In those instances in which an oncometer was placed around the kidney, injection of ½ cc. of suspended rabbit

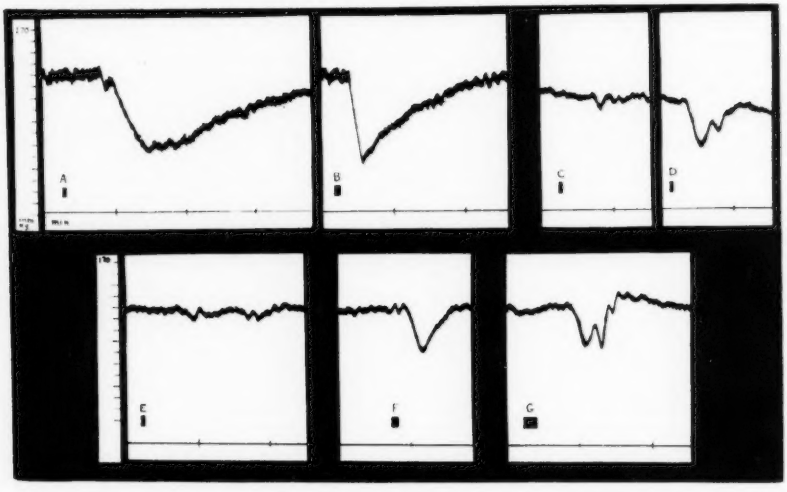

Fig. 2. Effect of evisceration upon vasodepressor effect of rabbit red blood cells. Carotid blood pressure in dog (no. 18). A, ½ cc. suspension of red blood cells. B, ½ cc. histamine, 1:10,000 dil. Between B and C, the intestines, spleen and pancreas were removed. C, ½ cc. suspension of red blood cells. D, ½ cc. histamine, 1:10,000 dil. Between D and E, the liver was removed. E, ½ cc. red blood cells. F, 3 cc. red blood cells. G, ½ cc. histamine, 1:10,000 dil.

red blood cells always resulted in a drop in volume coincident with, and parallel in magnitude to, the drop in blood pressure (fig. 1-1). As in the case of the limb, these observations indicate that any vasodilatation, if present, must have been masked by the passive changes caused by the drop in general blood pressure.

4. *Effect upon the heart.* In two experiments electrocardiograms were taken during the actual drop in pressure produced by the injection of suspended red blood cells. In each instance there was a change in the contour and direction of the T wave in at least two leads, without a significant change in heart rate (even when the drop in pressure was as much

as 100 mm. Hg). These changes are similar to those elicited by histamine in sufficient concentration to produce a comparable depression in blood pressure (1 cc. of 1:10,000 dil.). The T wave changes were, therefore, probably due to temporary anoxemia of the myocardium accompanying the depressor response.

These results indicate that the depressor action of rabbit cells in the dog is primarily on the peripheral circulation and not on the heart. A dilatation is produced in the splanchnic bed, and to a lesser extent in other regions, which acts on the heart indirectly 1, by decreasing the coronary flow (associated with the drop in blood pressure), and 2, by decreasing the venous return to the heart (associated with pooling of blood at the periphery). The latter mechanism is indicated by the finding of a drop in venous pressure concomitant with the drop in arterial pressure.

IV. Mode of action: *Stability of depressor substance.* In a number of instances the effect of exposing rabbit red blood cells to different temperatures for periods of 24 hours or more was determined. Neither incubation in the ice box nor at 37°C. had any effect on the vasodepressor property of the intact cell; the response being the same as that elicited by control bloods exposed to room temperature for no longer than one hour.

Difference between depressor response and anaphylactic state. There is very little similarity between the response produced by the injection of intact red blood cells and that resulting from the administration of an antigen to a sensitized animal. In the first place, the dogs used in these experiments had not been previously sensitized to the rabbit cells. Secondly, repeated injections continued to elicit depressor responses, which condition is different from that observed in a surviving shocked animal, for the latter generally becomes either completely refractory to any subsequent administration of the antigen (12), or the reaction is greatly modified.

Identification of the depressor substance in the intact red blood cell. 1. It is not acetylcholine. In each of two experiments, acetylcholine ($\frac{1}{2}$ cc. 1:60 dil.) was administered intravenously followed by atropine sulphate (1 cc. 1:1000 dil.). Then $\frac{1}{2}$ cc. of suspended rabbit red blood cells was injected and again the initial dose of acetylcholine. In each instance the depressor action of acetylcholine was prevented by atropine sulphate, while the response to the administration of blood was unaffected.

2. It is not histamine. If histamine were the substance in the intact red blood cell responsible for the vasodepressor effect, incubation of the cells with histaminase should abolish this property, since the latter enzyme specifically destroys histamine. On two occasions, rabbit red blood cells were incubated with 2 units of histaminase[2] for 36 hours, and in both

[2] One unit of histaminase is the quantity capable of detoxicating 1 mgm. of histamine in vitro during 24 hours at a temperature of 37°C.

instances subsequent injection of the mixture into the intact anesthetized dog demonstrated no decrease in the vasodepressor effect. Control mixtures of histamine (1:5,000 dil.) and 1 unit of histaminase, to which dog blood was added, manifested no depressor effects. The dog blood used in the control mixture demonstrates that there is no substance present in blood capable of inhibiting the action of histaminase. Further supporting evidence is the fact that hemolyzed red blood cells do not elicit this depressor effect. If histamine were the cause for the reaction, then the process of laking should not have prevented the depressor response, since histamine is not affected by such a procedure. This is easily demonstrated by adding it to dog cells and then laking the mixture; a drop in pressure is still elicited under these conditions. Further, it has been shown above that the time course of the reaction produced by the intravenous injection of red blood cells differs from that produced by histamine.

Correlation between in vivo and in vitro results. In order to obtain further information concerning the basis for the depressor effect, an attempt was made to determine whether or not any reaction took place in vitro between the red blood cells of the rabbit and the serum of the dog. A constant quantity (1 cc.) of a 2 per cent saline suspension of washed red blood cells was placed in a series of tubes and gradually diminishing amounts of diluted dog serum were added to each tube. The mixture was made up to 2 cc. with physiological saline and incubated at 37° for 45 minutes. The tubes were then centrifuged and examined for the point of first hemolysis. In every instance hemolysis was found, generally in dilutions as low as 0.06 to 0.03 cc. of serum. In a number of experiments the dog serum was first inactivated by heating to 56°C. for 30 minutes in order to destroy complement. The addition of rabbit red blood cells to this modified serum consistently resulted in no hemolysis, indicating that complement plays a necessary rôle in this hemolytic reaction. Furthermore it appears that a hemolysin is present in dog serum which affects rabbit red blood cells. That it is apparently heterologous is indicated by the finding that hemolysis is produced with cells from other species of animals (sheep, cat). This is in accord with the observations of Moss (13).

Illustrative of the close relation between in vivo and in vitro results are the following experiments. In two cases plasma was obtained from the dog and half of the amount was inactivated by the above method. To each portion, rabbit red blood cells were added ($\frac{1}{2}$ cc. of cells to 5 cc. plasma) and the mixture incubated at 37°C. for $\frac{1}{2}$ hour. At the end of this period, both tubes were centrifuged and, as was anticipated, the one containing the untreated serum showed marked hemolysis, while in the other, the supernatant fluid was clear. Each mixture was then injected separately into an anesthetized dog and the blood pressure recorded.

In the case of the mixture of cells and untreated serum, no effect upon blood pressure was observed (presumably because complete hemolysis had taken place), while the second mixture containing the inactivated serum produced a drop in pressure equal to that obtained with ½ cc. of a suspension of rabbit red blood cells. (All injections were made in a splenectomized dog in order to work with a consistently responsive animal.)

In a number of experiments the plasma of the dog was grossly examined for changes in color after 3 or 4 injections of ½ cc. quantities of rabbit red blood cells had been made, in order to determine whether hemolysis occurs in the blood stream as well as in the test tube. In practically every instance, if care was taken in obtaining the blood, no free hemoglobin was observed. Since the possibility existed that hemolysis of such a relatively small amount of rabbit cells, even if present, might not be sufficient to color the dog plasma, it was determined to inject much greater quantities of blood in the following manner:

The suspended washed red blood cells were administered intravenously by means of a constant injection pump which delivered the first 5 cc. over a period of about 30 to 35 minutes, the systemic effects being practically eliminated under these conditions. Thereafter, the rate of injection was slowly increased as the animal evidently entered the "refractory" state, and in this manner as much as 70 to 80 cc. of rabbit red blood cells could be administered over a period of 2 to 3 hours. Examination of the dog plasma about an hour after the completion of the injection revealed marked coloring (indicating hemoglobin in solution), this being present to a slight degree even after 24 hours. These findings show that hemolysis of rabbit red blood cells takes place just as readily in the animal as in the test tube.

In order to test the view that the depressor substance is liberated only when a specific red blood cell, like that of the rabbit, is acted upon by a hemolysin of the dog plasma, it appeared necessary to determine whether hemolysis of some other foreign cell in the blood stream could produce the reaction. Therefore, an attempt was made to strengthen artificially an already existing, but weak, heterohemolysin for cat cells in dog plasma in the following manner:

A dog (no. 19) was operated upon aseptically and a carotid artery cannulated in order to obtain carotid blood pressure readings. Both rabbit and cat cells were injected, with a resulting drop of 100 mm. in the case of the former and, as expected, no response with the latter. The carotid artery was tied off, the wounds were closed and the animal permitted to recover. In vitro experiments at this time demonstrated hemolysis of rabbit and cat red blood cells in 0.06 cc. and 0.7 cc. of dog serum respectively. Then at intervals of 2 days, gradually increasing doses of sus-

pended cat cells (beginning with ½ cc.) were injected intravenously. No symptoms were apparent even when 4½ cc. of cells were administered at one time. Four days following the 9th and last injection, a femoral artery was cannulated and again a drop of 100 mm. Hg was obtained with the injection of rabbit cells and no response with cat cells. In vitro experiments with blood obtained from the dog at this time revealed that cat cells were hemolyzed in a dilution of 0.04 cc. of dog serum as compared with one of 0.7 cc. at the beginning. The quantity of serum necessary for rabbit cells was also 0.04 cc. As a control, a similar type of experiment (dog 37), using rabbit cells instead of cat cells, was performed. Neither the in vivo nor the in vitro responses showed any significant changes when compared with the results obtained before the series of injections was begun. These experiments demonstrate clearly, therefore, that the mere reaction between cat red blood cells and a hemolysin (equal in concentration to that for rabbit cells) in the plasma of the dog is not sufficient to elicit the depressor response.

Discussion. An examination of the pertinent findings in the above experiments may be of help in formulating a feasible explanation for the depressor phenomenon. The first observation of importance is that intact red blood cells of certain species of animals are capable of producing a drop in pressure only when injected into a proper test animal, i.e., introduction of any foreign red blood cell into the blood stream of any animal is not sufficient to elicit the reaction. It is, therefore, necessary to assume either that the effective red blood cells per se are responsible for the drop in pressure, (the recipient playing a passive rôle), or that the test animal itself produces some change in the injected intact red blood cell which liberates an active depressor substance. Secondly, the relatively long latent period between the time of injection and the onset of the response, as compared with histamine, suggests that a reaction must take place in the blood stream of the recipient animal before the depressor effect can be manifested. Thirdly, since the response is transient in nature, it follows that the depressor substance is rapidly destroyed. Fourthly, in view of the correlation existing between in vivo and in vitro experiments, as well as the finding of free hemoglobin in the blood stream of the dog on injection of rabbit red blood cells, it might be inferred that the liberation of the depressor substance from the intact cell is in some manner bound up with the interaction taking place between the foreign cell and the plasma of the recipient. Finally, it follows that in the case of the rabbit cell, the heterologous hemolysin and the complement present in the dog are important only in that they supply the mechanism by means of which the cell can be destroyed in the blood stream. The exact nature of the short-lived vasodilator substance, thus released, has not been determined.

SUMMARY

The effect of the intravenous injection of heterologous blood was investigated in a series of 43 dogs, 6 cats and 3 rabbits. It was found that a drop of blood pressure generally resulted; this was especially marked when rabbit blood was injected into the anesthetized dog.

The vasodepressor effect of rabbit blood was found to be due to the intact red blood cell alone, the constituents of the cell itself (liberated by laking) as well as the other portions of the blood being ineffective.

Evidence is presented to show that the vasodepressor effect is not due to histamine or acetylcholine present in the red blood cell.

With repeated injections of rabbit cells, the drop in pressure becomes less and less until the animal enters a "refractory" state. Previous splenectomy either eliminates or modifies the appearance of the "refractory" state.

The drop in blood pressure appears to be due to vasodilatation of vessels especially in the splanchnic region and to a less extent in other areas.

From the evidence obtained it can be stated that intact rabbit red blood cells, when introduced into the blood stream of the dog, initiate a reaction involving hemolysis of the foreign cells, during which a short-lived vasodepressor substance is released. The mere hemolysis of any foreign cell, however, (as for instance, that of the cat) in the blood stream of the dog is not sufficient to produce the effect.

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THE DISTRIBUTION AND RATE OF DISAPPEARANCE OF INTRAVENOUSLY INJECTED HISTAMINE IN THE RAT

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The liberation of histamine by the organism into the blood stream under various conditions such as cell destruction, nervous stimulation or the anaphylactic reaction has been well established by the work of various authors (Bartosch, Feldberg and Nagel, 1932) (Feldberg and Kellaway, 1937) (Code, 1938). The subsequent fate of this liberated histamine has not been well understood. According to Feldberg (1937) histamine liberated by cell damage or by nervous stimulation goes into the tissue spaces and there exerts its action for a time. The transfer of the histamine from these spaces to the blood stream seems to be a slow process and the organism is therefore protected by this means from the general systemic effects of histamine although the local action may be prolonged.

MacGregor and Peat (1933) have shown that the isolated lung-kidney preparation, using the dog, is able to inactivate large amounts of histamine added to the perfusing fluid and that ten times the normal histamine content of dog lung so added could be inactivated in five minutes by the blood itself.

Code (1937b) has shown that the major part of the blood histamine is held in combination in the white blood cells, and more recently (1938) has demonstrated that in the anaphylactic reaction in the dog, the larger part of the liberated histamine is free in the plasma for a short time.

It seemed of interest to study further the fate of this liberated histamine in the intact animal. For this reason, experiments were performed in which the distribution and rate of disappearance of histamine were followed after the intravenous injection of this substance.

METHODS. The rat was chosen because this species will withstand large doses of histamine. The animals were of a hooded strain. They were first anesthetized with ether, the jugular vein was exposed and a solution of histamine in 0.85 per cent sodium chloride was injected intravenously.

[1] Aided by a grant from the Banting Research Foundation, Toronto, Canada.

The amount injected varied according to the weight of the animal, and the total volume injected never exceeded 2 cc. Following this injection, the animal was allowed to recover from the anesthesia. After a definite period, it was again anesthetized, the abdomen opened and a sample of blood was removed from the inferior vena cava. The tissues were then removed as rapidly as possible and placed in tared weighing flasks containing a 10 per cent solution of hydrochloric acid. The flasks were again weighed before beginning the digestion of the tissues.

Blood histamine was determined by the method of Barsoum and Gaddum (1935) as modified by Code (1937a). Tissues were extracted accord-

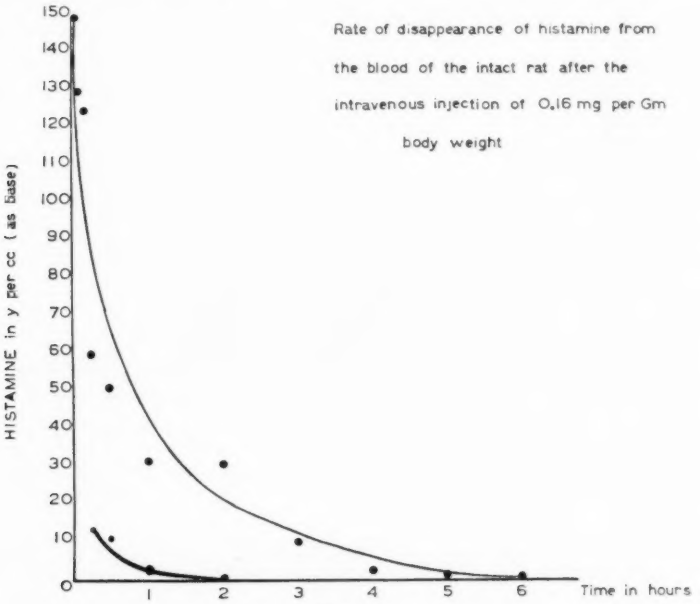

Fig. 1

ing to the method of Best and McHenry (1930). All assays were carried out on the isolated guinea-pig ileum preparation suspended in Tyrode's solution to which atropine was added in a concentration of one in ten million. Some of the extracts were checked using the blood pressure of the atropinized cat under Dial and chloralose anesthesia as the criterion. All values expressed throughout are given as histamine base.

Experimental results. a. *Intact animals.* The following preliminary experiment was performed in order to determine the rate of disappearance of histamine from the blood of the normal rat. Male animals weighing 250 grams were injected intravenously with a dose of 96 γ of

histamine per gram body weight. The total amount injected would then be 24,000 y (40 mgm. of histamine HCl, the salt used throughout the experiments). The animals were killed 3, 5, 10, 15, 30 minutes, 1, 2, 3, 4, 5,

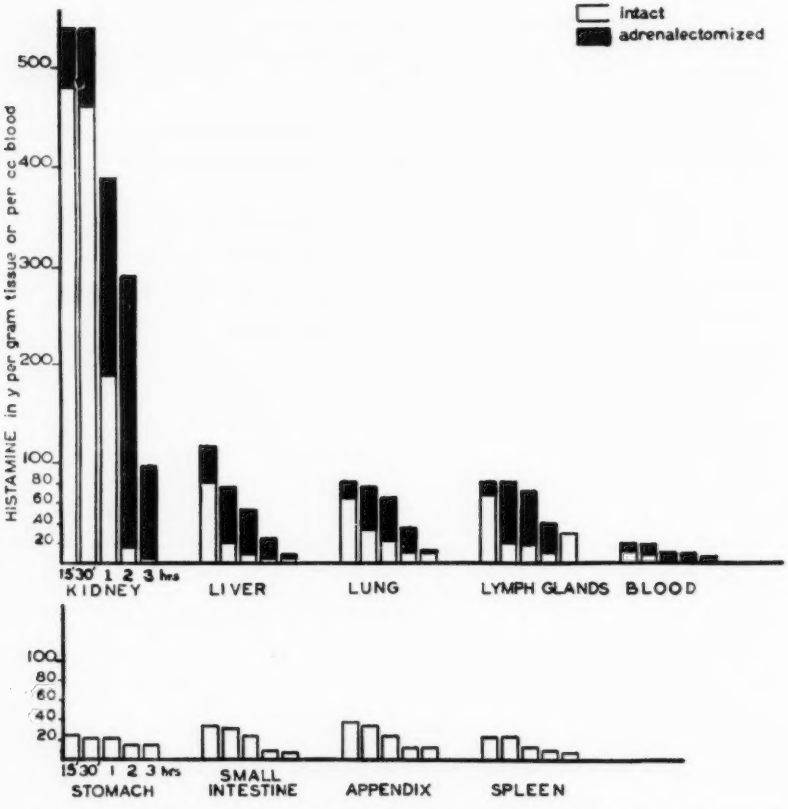

Fig. 2. Distribution and rate of disappearance of histamine from the tissues of the rat at given time intervals following the intravenous injection of 24 gamma of histamine (base) per gram body weight. The height of the white columns denotes the amount found in the tissues of the intact animal. The height of the black columns denotes the amount in gamma per gram found in the tissues of the adrenalectomized animal at the same time period. The abcissa represents time after the injection and is the same for each tissue.

and 6 hours after the injection had been given. The resulting curve is seen in figure 1. It should be noted that each point on the curve represents the determination on a single animal.

It will be seen that the value for the blood is 150 y per cc. three minutes

after the injection. Assuming that such an animal has a blood volume of 12 cc., even if no decrease of blood volume has taken place, the total histamine contained in the blood at that time would be 1800 γ or only about 7.5 per cent of the amount originally injected. As will be seen, the histamine content of the blood falls rapidly thereafter. At six hours, however, there is still some of the added histamine present in the blood, the value being 2.0 γ per cc. (normal value being about 0.05 γ per cc.).

From the foregoing preliminary experiment, it is evident that intravenously injected histamine disappears rapidly from the blood of the rat. In order to establish what the fate of this histamine might be, it was decided to study the tissue histamine content as well as that of the blood. Since the adrenalectomized rat is less resistant to histamine than the intact animal, and since it was desired to study the effects of adrenalectomy, a smaller dose of histamine was used throughout the subsequent

TABLE 1*

TIME	BLOOD		KIDNEY		LUNG		LIVER		LYMPH GLAND	
	Normal	Adrenal-ecto-mized	Normal	Adrenal-ecto-mized	Normal	Adrenal-ecto-mized	Normal	Adrenal-ecto-mized	Normal	Adrenal-ecto-mized
hours	*γ/cc.*	*γ/cc.*	*γ/gram*	*γ/gram*	*γ/gram*	*γ/gram*	*γ/gram*	*γ/gram*	*γ/gram*	*γ/gram*
¼	13.2	20.0	480	540	64	80	79	115	68	80
½	9.7	18.0	460	540	34	75	21	74	20	80
1	3.0	12.0	188	388	22	65	8	53	18	70
2	0.5	10.0	17	290	10	35	4	25	10	40
3	0.2	7.0	4.0	95	10	12	4	7	30	30

* The figures in table 1 have been revised from those published in the preliminary communication (Rose and Browne, 1938) in which table a number of the values were incorrectly reported.

experiments, namely, 24 γ histamine per gram body weight. The animals used in these experiments were all females weighing from 180 to 220 grams. In such an animal, the average dose of histamine injected was about 4800 γ (8.0 mgm. histamine HCl).

The histamine was injected in the same manner as described in the foregoing experiments, but in this series the animals were killed fifteen, thirty minutes, one, two and three hours after the injection. The tissues studied were blood, lung, liver, kidney and lymph glands. Later, data on the stomach, small intestine, spleen and appendix were added for the intact animal. The results are expressed in table 1 and figure 2. Each column represents the average of four to six experiments for normal and adrenalectomized rats. It will be observed from these results that even at fifteen minutes, the distribution of the histamine is markedly in favor of the tissues as compared with the blood. The latter showed a value

of 14.0 γ per cc. or about 280 times the normal. The histamine content of the lung was 64 γ per gram whereas the kidney histamine content was far in excess of that of either lung, liver or any of the other tissues studied, being 480 γ per gram or about 1600 times its normal value.

The histamine content of the other tissues, namely, stomach, small intestine, appendix and spleen was relatively only slightly raised, the increase never being greater than 20 times the normal amount fifteen minutes after the injection.

In considering the rate of disappearance of histamine from the tissues, it will be noted that during the fifteen to thirty minute period, the concentration of histamine decreases sharply in the liver, less markedly in the lung, blood or lymph glands. In sharp contrast to all the other tissues the concentration in the kidney decreases but slightly or not at all. In

TABLE 2

TISSUE	AVERAGE WEIGHT	AVERAGE HISTAMINE CONCENTRATION NORMAL	TOTAL HISTAMINE CONCENTRATION NORMAL	HISTAMINE CONCENTRATION 15 MINUTES AFTER INJECTION	TOTAL HISTAMINE CONCENTRATION 15 MINUTES AFTER INJECTION	DISTRIBUTION OF RECOVERED HISTAMINE 15 MINUTES AFTER THE INJECTION	PERCENTAGE RECOVERED AT 15 MINUTES OF TOTAL AMOUNT OF HISTAMINE INJECTED
	grams	*γ/gram or /cc.*	*γ*	*γ/gram or /cc.*	*γ*	*per cent*	
Blood	10 cc.	0.05	0.5	14.0	140	9.1	2.91
Lung	1.5	4.0–10.0	6.0–15.0	65.0	97	6.3	2.0
Liver	4.5	2.0– 4.0	9.0–18.0	80.0	360	23.4	7.5
Kidney	1.5	0.0– 0.4	0.6	480.0	720.0	46.7	15.0
Spleen	0.7	2.0– 5.0	3.5	25.0	17.5	1.15	0.36
Small intestine	4.5	5.0– 6.0	27.0	30.0	135	8.75	2.81
Appendix	0.8	10.0	8.0	38.8	31	2.1	0.66
Stomach	1.8	9.0	16.2	22.0	39.6	2.5	0.82

the thirty minute to one hour period, however, there is a rapid decrease in the histamine content of the kidney, from 460 γ per gram to 188 γ per gram. At the end of three hours, all values of the tissues approach normal with the exception of those of the blood and kidney which are still raised to some ten times the normal. If the histamine content of all the tissues studied is totalled at the fifteen minute period, 1540 γ or 32 per cent of the injected histamine is accounted for of which 720 γ are in the kidney (see table 2).

b. *Adrenalectomized animals.* Experiments were carried out on adrenalectomized animals under the same conditions as described in the foregoing experiments. Rats from the same colony as the above weighing from 180 to 220 grams on the average were adrenalectomized in the usual manner, given 0.85 per cent saline to drink and fed on a standard Purina

diet. Only animals in good health were used. The dose of injected histamine was the same, namely, 24 γ per gram body weight. The animals were killed at the same time intervals as those in the above group, that is, fifteen, thirty minutes, one, two and three hours after the injection of histamine. These animals will tolerate such a dose of histamine, although not as well as the intact animal.

In preliminary experiments, animals were used five to seven days after the adrenalectomy, and in this case, no definite change in the distribution of the histamine or in the rate of its disappearance from the blood or tissues could be noted. In the subsequent experiments, animals which had been adrenalectomized at least ten days were used. The results are shown in figure 2.

It will be noticed that at fifteen minutes after the injection of the histamine, the distribution of the histamine is in general the same as that for the intact animal. The blood histamine content however is higher even at fifteen minutes, it being 20.0 γ per cc. as compared with that of the intact animal which is 14.0 γ per cc.

In the subsequent time periods, it will be observed that there is a retardation in the rate of disappearance of the injected histamine from the blood and all the tissues. This retardation is definite at the thirty minute period and is most marked at one hour. Of all the tissues studied the kidney is most affected. At the three hour period, all of the tissue values have again approached normal with the exception of that of the kidney which is still 200 times the resting normal amount and that of the blood which is 100 times the normal amount. The stomach, small intestine, appendix and spleen were not studied in the adrenalectomized animal.

Discussion. The presence of a protective mechanism against the action of histamine has been postulated and has received support from the results of many workers. Best (1929) and later Best and McHenry (1930) demonstrated the presence of an enzyme which is capable of inactivating histamine in vitro. It was found to be present in the greatest quantities in the kidney of most species, but was not present in the kidney of the rat (McHenry and Gavin, 1931). This absence has been confirmed in this laboratory.

The experiments of Weiss, Ellis and Robb (1929), and of Jacobs and Mason (1936), indicate that small amounts of histamine injected intravenously at a slow rate produce none of the effects usually observed. This would seem to indicate that the blood is able to inactivate small amounts introduced into the blood stream at a slow rate. That this is not a histaminase-like action is shown by the experiments of Anrep, Barsoum and Talaat (1936) who demonstrated that histamine added to dogs' blood is in part taken up by the red blood cells and is thereby rendered physiologically inactive. If such blood is extracted for its histamine content,

the added histamine may be recovered. This has been greatly clarified by the recent work of Code (1938). In the dog, Dragstedt and Mead (1935) showed that intravenously injected histamine disappears very rapidly. They assayed the plasma only. Further evidence that this is not a histaminase-like action in the human at least is shown by the experiments of Yen and Chang (1933).

In other experiments done in this laboratory (in collaboration with Dr. S. Karady) it has been shown that there is no histaminase in any of the tissues of the rat with the exception of the lung and small intestine. In the experiments of MacGregor and Peat (1932), histamine was excreted by the kidney of the preparation. In the experiments which we have done at this time and in many others of a similar nature carried out in this laboratory, no urinary secretion has ever been observed in rats within three hours of the injection of such a dose of histamine.

Two possible explanations suggest themselves for the difference in the rate of disappearance of histamine from the various tissues studied. Assuming the presence of some other mechanism in the kidney than histaminase, the fall of the concentration in the lung, liver etc., during the fifteen to thirty minute period and the absence of any decrease in the kidney is due to the continued transfer of the histamine from the other tissues to the kidney at such a rate that the destruction of histamine by the kidney is not apparent.

If however one assumes that the main mechanism of histamine destruction is the histaminase action of those tissues which contain it, namely, lung and small intestine, the kidney in this case could be regarded as an organ which takes up a large amount of histamine, thus temporarily removing it from the circulation and slowly liberating it to be transferred to the histaminase containing organs for destruction. Under such circumstances, in view of the large amount of histamine disappearing from the kidney after the thirty minute period, one might expect the histamine concentration of the blood to remain relatively constant. On this basis, it is also difficult to understand why the liver, which contains no histaminase, should lose its histamine content in the period fifteen to thirty minutes, whereas that of the kidney should remain constant.

Rats which have been adrenalectomized are much less resistant to histamine than is the intact animal (Wyman, 1928) (Marmorston-Gottesman and Perla, 1931). In the experiments of the latter workers, the loss of resistance to histamine appeared only seven days after the removal of the glands. They were able to show that this resistance was due mainly to the presence of the cortex, since normal resistance was restored to the adrenalectomized animals by the administration of cortical extract. Ingle (1936) has shown that the medulla also plays a rôle.

The importance of the adrenal gland in the mechanism of the resistance

of the organism against the actions of histamine has been confirmed by our experiments. The decreased resistance to histamine however is not regarded as evidence that the specific function of the adrenal is to render possible the destruction of histamine. We rather regard the decreased ability to destroy histamine which develops only seven to ten days after adrenalectomy as a part of the generally decreased resistance of the organism under these conditions which is evident even when the animals are maintained in apparently good health on sodium chloride solution (Selye, 1937).

It would seem therefore that histamine which may be liberated into the blood stream may be dealt with in one of several ways, and that the absorption of the largest amount of this liberated histamine by the tissues, in particular the kidney, is one of these in the rat.

The amounts of histamine used in these experiments are larger than those which could in all probability be liberated under physiological or pathological conditions. However, using much smaller amounts, 1.2 γ per gram body weight, we have found that there is still a definite rise in the histamine content of the kidney, and further experiments on the distribution of these smaller amounts are in progress.

SUMMARY

1. The distribution of histamine injected intravenously into the intact and adrenalectomized rat is markedly in favor of the tissues as compared with the blood within fifteen minutes.

2. Of the tissues studied, the kidney takes up most histamine.

3. Although the concentration of histamine in the blood, lung, liver and lymph glands has begun to decrease within fifteen minutes after the injection, no apparent decrease is evident in the kidney up to the thirty minute period. After this time the rate of disappearance is greatest in the kidney.

4. Adrenalectomy has little effect on the initial tissue distribution of histamine injected intravenously in the rat, but markedly retards the rate of its disappearance. This effect is most marked in the kidney.

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QUANTITATIVE OBSERVATIONS ON THE EXTRA-VAGAL COMPONENT OF CAROTID SINUS PRESSORECEPTIVE CARDIAC RESTRAINT[1]

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It is accepted that in the chronotropic cardiac restraint arising from carotid sinus pressoreceptors, reflex excitation of the vagal inhibitory mechanism plays the major rôle. Experiments in which the pressoreceptors' activity has been altered by natural means (endovascular pressure changes) indicate that there is also a minor *extra-vagal* efferent influence (Hering, 1927; Heymans, Bouckaert and Regniers, 1933; Koch, 1931; Tournade, 1930; Schneyer, 1935; Winder, 1937).[2] Electrical records from the cardiac accelerator nerves evidence their participation in this minor rôle; that is, concurrent with reflex vagal excitation there is reciprocal inhibition of sympathetic cardio-accelerator influence (Bronk, Ferguson and Solandt, 1934; Bronk, Ferguson, Margaria and Solandt, 1936; Govaerts, 1936; Rijlant, 1936). In addition, it appears established that there is a carotid pressoreceptive reflex inhibition of epinephrine secretion occurring under experimental conditions (Heymans, Bouckaert and Regniers, 1933; Hering, 1929; Goormaghtigh and Elaut, 1929; Tournade, 1930; Aomura, 1930; Hartwich and Hessel, 1931; von Euler and Liljestrand, 1934), and in view of the mass-influence of the pressoreceptors on the sympathetic nervous division (Hering, 1932) a possibility of effective changes in circulating sympathin cannot be ignored. There are suggestions that such humoral elements taken together may operate measurably within the extra-vagal component of carotid pressoreceptive reflex cardiac restraint (Abe, 1936; Winder, 1937). Consequently, the term *extra-vagal* cardiac restraint must tentatively include reflex inhibition of cardio-accelerator nerves and reflex depression of humoral accelerators.

[1] These experiments were supported in part by a grant from the Rockefeller Foundation to Robert Gesell for studies on respiration.

[2] The inconsistent results of Inaba's (1931) study of the extra-vagal component appear attributable to the use of artificial electrical excitation of the mixed sinus nerve. In spite of the inherent deficiencies in that technique Code (1935) apparently obtained with it consistent evidence of the extra-vagal component, and Abe (1936) fairly consistent evidence. The "amphotropism" concept of Danielopolu appears untenable when applied to carotid pressoreceptive influence (Winder, 1937).

PURPOSE AND METHOD. The present note is concerned with the quantitative curve-form of reflex relationship between endosinual pressure and *total* extra-vagal chronotropic cardiac restraint. The observations were made by the technique described previously (Winder, 1938). In dogs anesthetized with morphine and urethane one carotid sinus was functionally excluded by common carotid occlusion; the other carotid bifurcation was vascularly isolated and the carotid body excluded by embolization; the isolated carotid sinus was perfused and aortic blood pressure controlled with blood circulating from the same animal; pulmonary ventilation was controlled during open pneumothorax; and the vago-sympathetic-aortic nerves were severed. *Static* endosinual pressure control could be readily substituted for the adjustable mean *pulsating* perfusion pressure. For each of these two types of pressure control two systems of pressure alterations were used: *a*, progressive *stepwise* increments from zero upward, and *b*, successively higher *rectangular* changes from and back to zero for each pressure level investigated. The pulse rate was counted from a mercury-manometer record of the controlled aortic blood pressure, just preceding each pressure change. In the various series the endosinual pressure was left at any one level for from 40 seconds to several minutes.

RESULTS AND DISCUSSION. The data are presented graphically in the figure. The changes in heart rate in terms of per cent of the rate obtaining at zero endosinual pressure are plotted against respective endosinual pressures. The basic or zero-pressure rate for each point is an interpolated value, located with reference to the rates at zero-pressures just preceding and first following the respective pressure level.

With either *static* or *pulsatile* endosinual pressure, the *rectangular* method of pressure changes resulted in considerable scattering of the plotted data of any one series of trials, although a certain trend was present each time. The individual points of seven series of such trials (six with ascending pressures and one with descending) made in three animals are indicated together at *A*. Mathematically smoothed graphic interpolation, grouping the variants of each successive 40 mm. Hg pressure range, yielded the mean trend shown at *B*. The results from one of these animals of a complete series of trials by the *stepwise* (static) procedure together with those of a more sketchy stepwise series (marked *2*) with correspondingly less time for attainment of steady conditions at each level, and the value for one prolonged rectangular pressure rise to maximal (marked *3*), all from the same animal, are shown at *C*. The concurrent pressoreceptive inhibition of breathing movements recorded plethysmographically (Winder, 1938) is shown at *D* for comparison. The data shown at *E* (rectangular procedure with static pressures) is from another of the three animals represented in *A* and *B*, and was taken without arterial pressure compensation and while the animal was constantly *over*ventilated to the extent

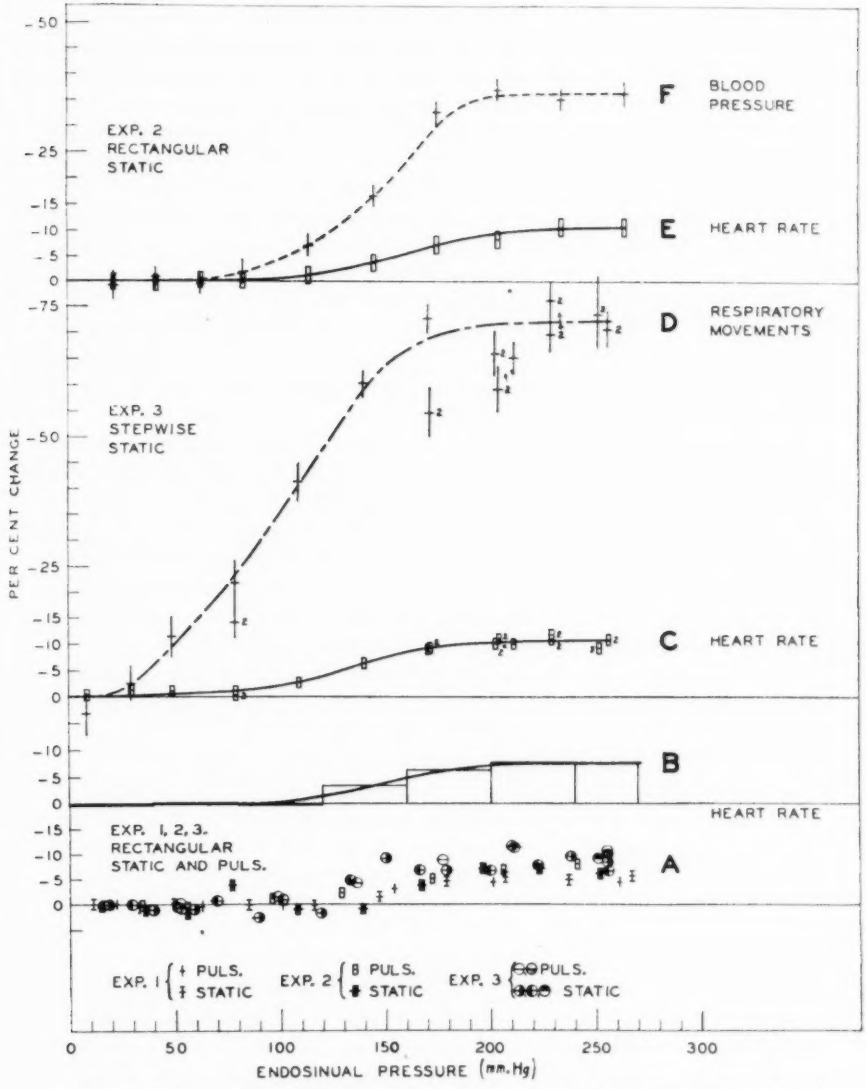

Fig. 1. Relationship between endosinual pressure (pulsatile or static, altered rectangularly or stepwise) and depression of heart-rate (in "vagotomized" dogs), respiratory movements and arterial blood pressure. During *A-B* and *C-D*, pulmonary ventilation and aortic blood-pressure controlled. During *E-F*, constant pulmonary *over*-ventilation to the extent of respiratory quiescence; aortic blood-pressure *un*controlled. Carotid body embolized.

of respiratory quiescence. The concurrent reflex fall in blood pressure is shown at *F* for comparison. A hypocapnic reduction in sympathetic activity probably provided less background of activity for depression of either heart rate or blood pressure than would have been the case otherwise.

The relative error in measurement of such small changes together with accidental scattering beyond the error of measurement made it impossible to determine accurately the threshold, turning point, and maximal endosinual pressures for the curve of reflex extra-vagal cardiac slowing. But apparently the sigmoid graphic course of relationship between endosinual pressure and extra-vagal slowing is completely parallel with that for total cardiac depression with vagus nerves intact as reported by Koch (1931) and Schneyer (1935), and with similar curves for vascular depression (Koch, 1931; Lim and Hsu, 1931; Schneyer, 1934) and respiratory depression (Winder, 1938) as evidenced directly by comparison of curves *C* and *D*, and *E* and *F*, respectively.

Alternative possible connections for these multiple end effects are: *a*, a common set of receptors and afferent paths having either parallel connections to the various central mechanisms involved, or primary connections with one or two and indirect inter-nuclear connections with the others (irradiation); or *b*, separate sets of receptors and afferent paths for the respective end-effects, with very similar or identical physiological characteristics. The first, implying multiple central influence of each individual pressoreceptor was early hypothesized by Kisch (Hering, 1932), and with current quantitative information is made even more probable. The conditions under which curves *E* and *F* were determined throw a small light on this question. The animal was constantly overventilated to the extent of respiratory quiescence; consequently, unless there can be central respiratory activity which does not reach the final motoneurones, it is indicated that there must be afferent connections with either or both the vasomotor or cardio-accelerator mechanisms, or with the integrated whole of which they are components—the sympatho-adrenal system—which are potentially independent of changes in activity of the respiratory mechanism.

If the probability of a common set of receptors becomes an actuality, then *excitation* of the vagal cardio-inhibitory mechanism with quantitatively parallel *inhibition* of vasomotor, cardio-accelerator and respiratory mechanisms would be a clear-cut case of operation of the law of reciprocal innervation, and, more peculiarly interesting, within a pattern of musculature at once of *autonomic* and *somatic* innervation.

If urethane anesthesia engenders or augments resting epinephrine secretion, then the approximately 8 per cent maximal slowing found in these experiments may represent some exaggeration. At best the extra-vagal component of cardiac restraint from one sinus is by itself small.

But if a resting accelerator influence to the heart furnishes a background which increases the efficiency of the vagal influence, then the observed small *variation* in that background in the useful direction may be of more importance (Bronk, Ferguson, Margaria and Solandt, 1936) than apparent from its isolated consideration.

In these experiments with arterial pressure and pulmonary ventilation controlled and any possible influence of altered carotid body circulation eliminated, the highest maximal cardiac slowing was 11 to 12 per cent, whereas in previous experiments without these measures of control, but with the same anesthesia, over 20 per cent slowing was sometimes seen (Winder, 1937). In one of the present experiments, yielding 7 to 8 per cent maximal slowing under controlled conditions, subsequently allowing natural control over ventilation by the concurrently inhibited breathing movements contributed 2 to 4 per cent additional slowing, and when further the severe reflex depression of arterial pressure was not compensated, a total additional maximal slowing of approximately 6 per cent was seen. The fundamental curve trend did not change and should not be expected to in view of the circumstance that breathing movements and arterial pressure are depressed in quantitatively parallel fashions. In several preliminary experiments in which the *carotid body* was *not embolized* a slight hump tended to appear in the early part of the curve of plotted heart rate depression. A similar hump was occasionally much more marked in the plotted curve of blood-pressure depression (with uncompensated arterial pressure) and still more marked for the respiration curve. It is in accord with the circumstances that under the conditions of dissection employed carotid body ischemic excitation must have occurred at near-zero intracarotid pressures (Winder, 1938; Winder, Bernthal and Weeks, 1938) to be removed during the first increases in pressure along with its feeble direct or indirect extra-vagal accelerator influence on the heart (Bernthal, 1938b), its pressor influence on the vascular system (Heymans, Bouckaert and Regniers, 1933; Bernthal, 1938a) and its well known excitatory influence on the respiratory mechanism. In other preliminary experiments in which special means were not used to eliminate unnatural tug on the carotids by the perfusion connections,there was a very early and sharp downward kink in the plotted curves, slight and occasional for heart rate, more marked and more frequent for blood pressure (when uncompensated) and respiratory movements (cf. Schneyer, 1934, for an explanation).

SUMMARY

The extra-vagal component (sympathetic and humoral) of carotid pressoreceptive chronotropic cardiac restraint has been studied quantitatively in dogs anesthetized with morphine and urethane. Major secondary factors were controlled by means of carotid body exclusion (embolization), constant aortic blood pressure and pulmonary ventilation and total vago-sympathetic-aortic denervation.

The curve relating endosinual pressure and extra-vagal heart slowing by a single carotid sinus was sigmoid, and parallel with those relating endosinual pressure to total (vagi intact) cardiac slowing, vascular depression and respiratory depression. The maximal slowing was approximately 8 per cent.

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THE ISOLATION OF SECRETIN

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Since a recognition of a hormone control of the secretion of pancreatic juice many attempts have been made to isolate the specific agent known as secretin. Such attempts have yielded very potent concentrates (1–15) and have led to the discovery of other endocrine activities of the intestinal mucosa. However, no one has thus far reported the isolation of secretin of unquestionable purity with the probable exception of Hammarsten, Wilander, and Agren (13–16).

We desire to report a procedure which has resulted uniformly in the isolation of a crystalline salt of secretion. This crystalline salt has the properties of a pure compound.

EXPERIMENTAL. *Method of preparation.* A modification of the method of Ivy and his collaborators (11) was used as a basis for further purification. Long experience has shown this method to be reliable for securing a uniform product which has been termed SI. By appropriate treatment of SI with aniline a large amount of impurity was removed, and further purification was effected by butyl alcohol extraction. The aqueous residue after butyl alcohol extraction was treated with picrolonic acid, which resulted in a crystalline precipitate of secretin picrolonate. This precipitate was recrystallized by solution in warm pyridine and precipitation with several volumes of ether. After 2 recrystallizations the product manifested a sharp melting point, and the results of its analyses provided confirmation of uniformity and purity.

The method of isolation in detail follows:

A. *Preparation of precipitate "A".* The first six feet of small intestine are obtained from freshly slaughtered hogs, and immediately washed and turned inside out. Three hundred and sixty such pieces are soaked in 72 liters of cold 0.4 per cent HCl for one-half hour with frequent stirring. Then the pieces are wrung out into the acid extract which is then thoroughly shaken with 21.6 kilos of sodium chloride (8). These operations are carried out at the slaughter house. The mixture is then brought to the laboratory and filtered by suction until it has the consistency of a stiff paste, which is precipitate "A".

B. *Preparation of SI.* Six hundred grams of precipitate "A" are triturated with a mixture of 2700 cc. of aldehyde-free 95 per cent alcohol and 300 cc. of water to obtain a uniform suspension. This is filtered and the residue is again extracted. The combined filtrates are mixed with water and vacuum-distilled at a low temperature (37°C. or lower) until all the alcohol is removed, with the occasional addition of water to keep the volume at about 2500 cc. The resulting turbid aqueous mixture is brought to a pH of 5.4 with NaOH (glass electrode) and filtered. (After adjusting the pH at 5.4, the application of heat improves flocculation and filtration.) Strong trichloracetic acid solution (6.1N) is added at once to the clear filtrate (room temperature) to obtain a final concentration of 5 per cent. The resulting precipitate is collected by centrifuging and dried by repeated washing with 1:1 mixture of absolute aldehyde-free acetone and ether. This yields the powder termed SI.

C. *Purification with aniline.* One gram of SI is dissolved in 20 cc. of redistilled water acidified with 1 drop of concentrated HCl. Absolute aldehyde-free acetone is then added until a faint permanent turbidity results or until the total addition is 80 cc. To this mixture 5 cc. of aniline (the aniline was redistilled over tin just before use) are added and then sufficient acetone to make the volume up to 105 cc. The mixture is stirred and then centrifuged. The precipitate is subjected twice again to the acetone-aniline treatment. The combined filtrates are poured into an equal volume of water, and the resulting turbid solution is vacuum-distilled, with the occasional addition of water to maintain the original volume, until drops of aniline no longer appear in the distillate. The solution is then evaporated to dryness under reduced pressure, and the residue is thoroughly extracted with absolute methyl alcohol. This suspension is filtered, the residue washed with absolute methyl alcohol, and the filtrate and washings are treated with five volumes of ether. The resulting precipitate is centrifuged and washed with ether until the washings, evaporated to dryness and dissolved in water, yield no color reaction for aniline when treated with potassium dichromate and sulphuric acid.

D. *Isolation of crystalline secretin picrolonate.* Fifty milligrams of the product obtained by treatment with aniline are dissolved in 75 cc. of redistilled water. This solution is extracted five times with 20 cc. portions of n-butyl alcohol, using centrifugable separatory funnels to secure prompt separation after extraction. The combined butyl alcohol extracts are washed twice with 25 cc. portions of water, the washings being combined with the original aqueous layer. This is filtered and then vacuum-distilled until free of butyl alcohol. The solution is then stirred with 250 mgm. of picrolonic acid in a little warm acetone. After the resulting precipitate settles and the supernatant liquid is tested for complete precipitation, the mixture is left in the refrigerator overnight and then filtered. The residue

consists of small needle-like crystals which melt with decomposition at 220–230°C. This product is air-dried and recrystallized by solution in warm (about 50°C.) pyridine, filtration, and precipitation with five volumes of ether. This yields a product which crystallizes in the form of fine yellow needles melting with decomposition at 234–235°C. Recrystallization may be repeated without loss of activity.

An aqueous solution of the free secretin base was prepared from the picrolonate by suspending the picrolonate in dilute sulphuric acid and repeatedly extracting with ether to remove all the picrolonic acid. The aqueous layer was carefully neutralized with barium hydroxide solution until the excess sulphuric acid was exactly removed. The base could not be unequivocally isolated in the dry state.

The method of preparation may be represented by the following diagram, with the intermediate steps designated by number for convenience in further discussion.

I. Acid extract of upper intestinal mucosa.
Salt saturation
II. Precipitate — Filtrate is discarded.
70 per cent alcohol extraction
III. Residue is discarded.
IV. Solution
Removal of alcohol and isoelectric precipitation
V. Aqueous solution — Precipitate is discarded.
5 per cent trichloracetic precipitation
VI. Precipitate is SI.
Treatment with acetone and aniline
Solution — Precipitate, VIII, is discarded
Removal of aniline, CH_3OH extraction, and ether precipitation
VII. Ether precipitate
N-butyl alcohol extraction and then picrolonic acid addition.
IX. Crystalline picrolonate recrystallized by solution in warm pyridine and precipitation with ether.
X. Base.

Method of assay. Dogs under pentobarbital anesthesia were used. A cannula was inserted into the chief pancreatic duct and the carotid blood pressure was recorded. The animal was then given several threshold doses of SI, fraction VI, to fill the cannula and tubing with juice for activation of the automatic drop recorder. After return to a basal flow, the pancreas of the animal was standardized to a preparation of secretin of known potency kept for this purpose. This standardized secretin was SI, or fraction VI, and 0.25 mgm. constituted a *dog unit* (11), as determined on ten dogs. Injections of the unknown preparations were then made, interspersed with an occasional restandardization to eliminate the possibility of spontaneous variations in the responsiveness of the animal.

An assay was considered adequate when a given weight of unknown elicited on at least two test injections the same amount of secretion as a given weight of standard in the same time interval. The potency of the unknown was then determined by calculating:

$\frac{\text{Weight of unknown}}{\text{Weight of standard}} \times 0.25$. Before any unknown preparation was given a definite value in units, it was assayed on a minimum of five dogs. Also, any assay was discarded in which the unknown caused a fall in blood pressure. These rather detailed remarks are made because reports from Hammarsten's laboratory (14) describe the isolation of a crystalline picrolonate of secretin which is active in a dose of 0.004 mgm. according to the method of assay they used. The unit as defined by Hammarsten's group is that amount of material which will give rise to sufficient secretion to neutralize to methyl red 0.1 cc. of 0.1 N acid; the cat was used.

The unit as defined for the dog by Ivy et al. (11) is that amount of dried material in solution which when injected intravenously will cause a 10 drop (0.4 cc.) increase in rate of flow of pancreatic juice within a ten minute period following the time of injection as compared with the preceding ten minute period. (The flow of the preceding ten minute period is, of course, a spontaneous basal flow, which rarely amounts to more than one drop every two minutes.)

Comparison of units of secretin. In order to compare the unit used by Hammarsten's group with the one used in our laboratory, assays were made on cats with the pancreatic duct cannulated. The actual cannulation of the pancreatic duct was considered by us to be obviously superior to that of merely collecting the mixed secretions which accumulate on the duodenal mucosa in the region of the ampulla of Vater, as practiced by the Swedish investigators (16). Titrations of 10 samples of cat's pancreatic juice obtained by direct cannulation revealed that 1 cc. required 1.32 cc. of 0.1 N acid to bring it to the neutral point of methyl red; this showed that 1 cc. of pancreatic juice is equivalent to 13.2 Hammarsten units.

Typical results of comparative assays of our products on cats are shown in table 1. Using our standard secretin SI in 6 tests, we find that 1 Ivy-dog unit is equivalent to 2 Ivy-cat units. When the same material is assayed on the cat, the Ivy-cat unit is found to be ten times that of the Hammarsten. Thus, since at least two times more of the same material is required to give a threshold dose in the dog than the cat, the Ivy-dog unit is approximately twenty times greater than the Hammarsten-cat unit.

Properties of secretin picrolonate. Secretin picrolonate is a yellow solid, crystallizing in clumps of fine needles, and is very sparingly soluble except in acetone-water mixtures and in solvents which decompose it. After

two recrystallizations (fig.1), it melts with decomposition at 234–235°C. It was assayed by suspending a weighted amount in dilute acid, and extracting the pierolonic acid with ether. By this method of preparing the pierolonate for injection, it was found that 0.075 mgm., or 0.080 mgm., constituted a dog unit and 0.004 mgm. a Hammarsten-cat unit.

The pierolonic acid recovered by treatment of the secretin pierolonate with acid and ether was found by weighing to comprise slightly more than 80 per cent of the weight of the pierolonate. The identity of the pierolonic acid was confirmed by melting point (decomp.) determinations.

Qualitative analysis of the pierolonate showed the presence of carbon and nitrogen. Halogen, sulphur, and phosphorus, which were present

TABLE 1

Results of comparative assay of secretin in the cat, or a comparison of the Hammarsten and Ivy units for secretin

	CAT 1	CAT 2	CAT 3
Cats were given 0.25 mgm. of our standard S.I., or 1 dog unit			
Response in drops of juice	25	18	28
Response in cc. of juice	1.2	0.9	1.4
Cat dosage in mgm. according to Ivy units*.	0.13	0.18	0.12
Cat dosage in mgm. according to Hammarsten units	0.015	0.021	0.013
Cats were given 0.08 mgm. of our crystalline secretin pierolonate			
Drops of juice	35	19	36
Cubic centimer of juice	1.7	0.9	1.7
Cat dosage in mgm. according to Ivy units*.	0.04	0.055	0.04
Cat dosage in mgm. according to Hammarsten units	0.0036	0.0057	0.0034

* Threshold dose obtained by direct assay, i.e., not calculated from the response to 0.25 mgm. or 0.08 mgm. of secretin.

in the crude preparations, were absent. Quantitative analyses of the recrystallized pierolonate were performed by two commercial analysts on different samples with the following results.

	I	II	III	IV
Carbon	51.92	52.11	51.53	51.82
Hydrogen	4.33	4.65	4.61	4.70
Nitrogen	20.04	20.10	19.80	20.06

A limited amount of investigation of the aqueous solution of free secretin prepared from the pierolonate revealed that it is weakly basic, an observa-

tion of significance in regard to the structure of the molecule. An Ivy-dog unit of the free secretin was found to be 0.014 mgm. A secretin hydrochloride (non-crystalline) made from the free secretin gave a bright blue color in response to the biuret test and negative tests to the other usual color tests for proteins (Millon, Ninhydrin, and Hopkins-Cole) and their derivatives. It was not oxidized by aldehyde reagents, but was readily

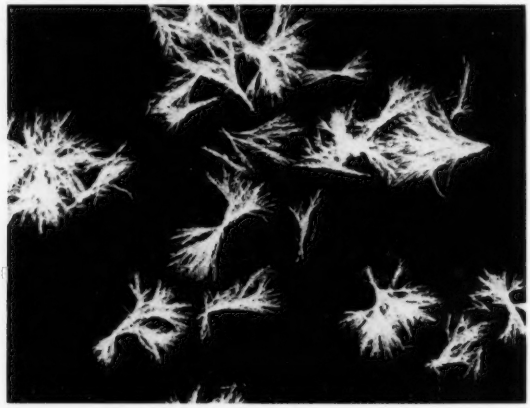

Fig. 1

TABLE 2

Yield of secretin activity from 100 pieces of the first six feet of hog's intestine

FRACTION	DOSAGE, DOG UNIT	YIELD, DRY WEIGHT	TOTAL DOSES DOG UNITS	YIELD OF TOTAL	VASO-DILATIN	ASH	TOTAL N	N.P.N.
	mgm.	*grams*		*per cent*				
I	11	336	30,000	100	++++	13.9	11.0	1.5
II	35	750	21,000	70	+	20.0	6.0	0.8
V	29	550	16,000	53	+	89.6	1.9	0.9
VI	0.25	2.8	11,000	37	0	0.4	17.5	3.8
VII	0.05	0.45	9,000	30	0	0	6.9	6.9
IX	0.080	0.5	6,000	20	0		20.1	
X	0.014	0.08	6,000	20	0			

oxidized on gentle warming with neutral potassium permanganate solution with complete destruction of its activity. The dry base did not react with either acetyl chloride or with methyl magnesium iodide in ether solution.

Yield. The yield of secretin activity from 100 pieces of the first six feet of hog's intestine expressed in dog units is shown in table 2.

Discussion. Deproteinization of the trichloracetic precipitate, or fraction V, was accomplished by acetone-aniline treatment. Insofar as

can be ascertained, this reaction represents a new method of deproteinization. The resulting secretin concentrate, after further purification with butyl alcohol, yields the product which crystallizes as the picrolonate.

The physical characteristics and sharp melting point of the crystalline picrolonate indicate its purity. The empirical formula of the picrolonate as calculated from the elementary composition is C_3H_3ON; thus:

CONSTITUENT	PER CENT	ATOMIC WEIGHT	RELATIVE NUMBER OF ATOMS
Carbon	51.8	12	= 4.32 ÷ 1.44 = 3.000
Hydrogen	4.5+	1	= 4.50 ÷ 1.44 = 3.120
Nitrogen	20.1+	14	= 1.44 ÷ 1.44 = 1.000
Oxygen (by diff.)	23.4+	16	= 1.46 ÷ 1.44 = 1.001

Secretin makes up only a small portion of the picrolonate, since approximately 80 per cent of the weight of the picrolonate consists of picrolonic acid. The small amount of secretin base at hand made accurate study of it impossible. The results of study of the little available indicate that it is very weakly basic, and that the nitrogen of its molecule probably does not exist as amino nitrogen. The results of other tests mentioned above would rule out the presence of carboxyl groups and reactive hydrogen atoms.

The potency of our secretin picrolonate compares very favorably with that of the Hammarsten group. The very low dosage, 0.004 mgm. per unit, reported by that group was obtained by their cat method. When we assayed our picrolonate by their method, we also obtained a cat-unit of 0.004 mgm., which in the dog amounts to 0.080 mgm. per unit. An interesting discrepancy exists, however, between our picrolonate and a statement they make concerning theirs. They believe that because of the large molecular weight (5000) they obtained, picrolonic acid can form only a small part of the molecule of their secretin picrolonate. We find that approximately 80 per cent of our picrolonate is picrolonic acid, which was checked by the fact that the free secretin base by direct assay had a dog unitage of 0.014 mgm. (or by calculation, a cat unitage of 0.0007) or approximately one-fifth the dosage of the picrolonate. They did not directly determine the picrolonic acid in their picrolonate, nor do they give the melting point of their product. This discrepancy is mentioned because it suggests the interesting possibility that their compound may have the same relation to secretin as thyreoglobulin has to thyroxin. This suggestion is supported by the observation that the secretin of the Hammarsten group can be digested with aminopolypeptidase and ten aminoacids split-off without a loss in activity (17).

The high content of picrolonic acid in our picrolonate indicates that we are dealing with a double salt; this and other studies on very small

amounts of the free base suggests that secretin is not a very complex substance. The determination of the structure of the molecule of secretin must await the isolation of much larger quantities of material than we have had available. An encouraging desideratum toward this end is the uniformity with which the crystalline picrolonate can be obtained. To date the results of the chemical study of the free base are equivocal.

SUMMARY

1. Secretin has been isolated in the form of a crystalline picrolonate. Eight-hundredths milligram of this compound constitutes an Ivy-dog unit, and 0.004 mgm. a Hammarsten-cat unit of secretin. Fourteen-thousandths milligram of the free secretin prepared from the picrolonate constitutes an Ivy-dog unit.

2. The crystalline picrolonate melts sharply with decomposition at 234–235°C. Picrolonic acid makes up most of the molecule, which possesses the empirical formula C_3H_3ON.

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THE IMMEDIATE EFFECTS OF THE OCCLUSION OF THE CORONARY VEINS ON COLLATERAL BLOOD FLOW IN THE CORONARY ARTERIES[1]

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The problem of encouraging blood flow into potentially infarcted areas of the heart is intriguing and has been approached from various angles. Studies from this laboratory have shown that the normal collateral circulation in the dog's heart (2) is insignificant, but that a large new intercoronary circuit can develop following chronic occlusion of any of the three major coronary rami (3, 4). The idea, based on anatomical studies, has also been advanced that a collateral circulation can be encouraged to develop by partial ligation of the cardiac venous system. For example, experiments of Gross (5) and Robertson (6) indicate that, following chronic coronary sinus or vein occlusion, prior to chronic central occlusion of a coronary artery, there is a marked reduction in the size of the infarct which follows chronic coronary occlusion alone and extra-coronary anastomoses appear. Fortunately, such anatomical observations can be tested physiologically and hence an experimental study was made of the possible practical value of such venous ligation in improving the collateral blood flow to potentially infarcted areas of the heart.

If such a procedure is to be of any great value to an area of the heart previously rendered ischemic by occlusion of a coronary artery the following conditions must be met: 1, the retrograde blood flow from the peripheral end of a centrally occluded coronary artery must be adequate in volume and contain sufficient oxygen to nourish the myocardium; that is, the minute-volume of oxygen supplied by new channels must approach that which would normally enter the myocardium when its normal supply of blood is unobstructed; and 2, myographic records, taken after both the coronary sinus and the coronary artery are occluded, must give definite evidence of the maintenance of contraction in the zone normally fed by the centrally occluded coronary artery. Finally, if these conditions are fulfilled, blockage of the source of the extra-collateral blood flow must reduce such flows to values that would be obtained after central coronary

[1] Preliminary reports of this work have been presented at the American Physiological Society Meeting in Baltimore, April 1, 1938 (1) and before the Society for Experimental Biology and Medicine in Cleveland, Ohio, April 7, 1938.

ligation alone, and must induce failure of contraction. Our experiments were designed to give evidence on these points.

METHOD OF STUDY. The experiments were performed on normal dogs anesthetized with morphine and sodium barbital and with the heart exposed. Coagulation was prevented by introducing into the circulation a combination of heparin (5 mgm. per kilo) and chlorozol fast pink (60–70 mgm. per kilo). Pressures were taken from the aorta by a cannula placed at the aortic valves and from the central and peripheral ends of the ramus descendens anterior or right coronary by insertion of a glass cannula into a side branch of the coronary artery. All pressure pulses were optically recorded by use of the Gregg optical manometer (7).[2]

Since the original publication certain changes have been made in the manometer, in the recording membranes and in the method of mounting mirrors, all of which reduce the chances for low frequencies to a minimum and eliminate the remote possibility of the appearance of hysteresis in the records. To facilitate detection of possible air bubbles trapped in the manometer the segment tips are detachable and both tips and barrel are made of transparent material (Lucite),[3] instead of Monel metal. Transparent or translucent tubing of a variety of types and which can be adjusted to any desired position has been substituted on some occasions for the lead tubing connecting the manometer barrel to the hypodermic needle or cannula. Special rubber stock with low hysteresis from 0.006 in. to 0.022 in. in thickness and stretched 3 to 7 times is used for the membrane. This rubber displays no hysteresis even when the applied internal pressure is 300 mm. Hg and the sensitivity is such as to give a deflection of the light beam of 15 mm./1 mm. Hg. Although for the usual pressure recording hysteresis is not likely to occur as the result of loose contact between membrane and mirror the use of very loose membranes will introduce a variable amount of it. This can be entirely eliminated through any one of several simple expedients: 1. The mirror can be mounted directly on the membrane by a good grade of self-vulcanizing cement or by adding sulphur chloride to the rubber cement. 2. The mirror can be mounted with ordinary cement on a small thin shaving of bamboo or hard rubber which in turn is attached to the membrane by self-vulcanizing cement, or it can be mounted on a small pedestal 1 mm. or so in diameter made of the same material, which is fixed to the membrane by a variety of compounds such as "Vulcalock,"[4] or self-vulcanizing

[2] This manometer was demonstrated before the American Physiological Society, April 23, 1937, at Memphis, Tenn.

[3] This material was obtained through the courtesy of E. I. du Pont de Nemours and Co., Arlington, N. J.

[4] We are deeply indebted to Dr. A. Szegvari of the American Anode Co., Akron, Ohio, for furnishing the special liquid rubber compounds and membranes and also for suggesting the various expedients mentioned above which eliminate hysteresis from the mirror mountings.

cement. 3. Pedestals can be made directly and in place out of latex molding compound.

The peripheral flow was measured by opening a side stopcock in the coronary cannula and collecting the blood in a micro-pipette or graduated cylinder. Determinations of flow were of course made before, during and after venous occlusion. In some experiments the retrograde flow was collected under oil and determinations of oxygen capacity and content made. Systemic blood pressures were elevated, when desired, either by injecting neosynephrine or by placing an adjustable clamp around the aorta just above the diaphragm. Blood volume was maintained by slowly infusing warm Locke's solution, 6 per cent acacia or homologous blood through the external jugular vein. The ability of the myocardium to contract when coronary sinus occlusion was added to central coronary clamping was tested by use of the myograph (8).

In an actual experiment the sequence of events was as follows: A central coronary was occluded for 0.2 to 0.3 minute while its peripheral pulse and the aortic pressure were recorded. The normal peripheral flow was then determined while aortic pressure and myograms from the area normally fed by the occluded coronary were recorded. This was continued (with or without the peripheral coronary being allowed to bleed externally) until the myogram showed systolic extension. The central end of the coronary artery was then released and a 5–10 minute recovery period allowed. The coronary sinus or great cardiac vein was then occluded, and, at various times (up to 4 hours) after its occlusion, the central coronary was reclamped and the same procedure repeated at various blood pressure levels. At different times in the same experiment the other coronary rami were clamped separately or together to determine their possible effect on the peripheral coronary flow, pressure and myogram.

Results. Twenty-eight successful experiments have been completed. Quantitation of the changes in retrograde flow in the ramus descendens anterior and the right coronary artery after venous ligation are set forth in typical experiments in table 1. Following ligation of the venous system the back flow in the ramus descendens anterior rises appreciably. For example, in experiment 2 after coronary sinus ligation the back flow rises from a normal control of 1.4 cc. per minute to 32 cc. per minute, while the aortic pressure remains at 127/100 mm. Hg. Upon release of the sinus the back flow returns to its normal control level. Back flows of this magnitude are also contained in experiments 6, 7 and 8. The extent of such flow augmentation varies however from dog to dog and in some experiments is much less. In experiments 4 and 5 the flows are 4.9 and 8.9 cc. per minute respectively. However, no experiment has been found in which sinus blockage did not increase the peripheral flow tremendously.

The effect of less drastic cardiac venous occlusion on retrograde flow has been studied in 6 experiments. In experiment 9 following occlusion of the

great cardiac vein the flow rises from 0.63 cc. per minute to 1.62 cc. per minute. Such mild elevation of the peripheral coronary flow after partial venous occlusion is the rule and typical of other experiments.

Although the majority of normal peripheral flows following acute occlusion of the ramus descendens anterior alone are similar to those previ-

TABLE 1

Effect of cardiac venous occlusion on peripheral coronary blood flow

EXPERIMENT	DOG WEIGHT	AORTIC PRESS.	CORONARY FLOW	CONDITION
			Right coronary	
	kgm.		*cc./min.*	
1	9.5	152/133	0.55	Cor. sinus open. Control
		152/117	1.05	Cor. sinus closed
			Ramus descendens anterior	
2	9.0	127/100	1.4	Cor. sinus open. Control
		127/100	32.0	Cor. sinus closed
		112/82	1.4	Recovery
3	12.0	90/62	2.4	Cor. sinus open. Control
		86/58	11.6	Cor. sinus closed
4	12.0	95/78	0.7	Cor. sinus open. Control
		82/52	4.9	Cor. sinus closed 41 min.
		85/52	1.96	Cor. sinus closed 42 min. Left circ. clamped
		85/60	4.90	Cor. sinus closed. Release left circ.
		90/62	4.34	Cor. sinus closed 60 min.
		88/66	2.94	Cor. sinus closed 65 min. Rt. cor. clamped
		90/72	4.20	Cor. sinus closed. Release rt. cor.
5	11.0	109/90	1.54	Cor. sinus open. Control
		104/87	5.40	Cor. sinus closed 2 min.
		109/94	8.96	Cor. sinus closed 51 min.
6	12.5	50 (mean)	6.0	Cor. sinus open 23 min. after clamp for 21 min.
		100 (mean)	32.0	Cor. sinus closed 25 min.
7	11.0	125/10	29.0	Cor. sinus closed 3 min. after previous closure of 25 min. and release
8	11.5	88/62	3.5	Cor. sinus closed 4 min.
		125/70	15.0	Central cor. also closed and 450 cc. blood infusion into coronary sinus at 180 mm. Hg pressure, 3 min.
9	13.3	69/28	0.63	Normal. Control
		69/33	1.62	Great cardiac vein clamped 13 min.

ously reported (1), that is, they are less than 1.0 cc. per minute, a goodly number are far in excess of such figures. For example, in table 1, experiments 3, 5 and 6, the back flows are 2.4, 1.54 and 6.0 cc. per minute. An inspection of all our control flow figures shows that such values appear

only after the coronary sinus has been previously occluded for some time and then released. This indicates that the augmentation of such collateral connections can persist for some time after venous occlusion has been removed.

An average of all our results on backflows, including many experiments not in table 1, shows that at aortic blood pressures ranging from 50/29 to 98/74 mm. Hg, the normal back flow is 0.91 cc. per minute, whereas with the coronary sinus also ligated the flow is 6.4 cc. per minute. At higher aortic pressures of 102/78 to 127/100 mm. Hg the normal back flow is 1.4 cc. per minute as contrasted with 14 cc. per minute after sinus ligation.

Although such increases in retrograde flow in the left coronary invariably follow cardiac venous occlusion such changes do not as a rule occur in the right coronary after its occlusion. In six experiments in which the right coronary and the sinus have been occluded we have only occasionally observed a very small elevation of the peripheral flow. This is of the order shown in experiment 1, table 1.

When coronary sinus ligation is added to central coronary occlusion the augmentation of back flow may reach a maximum at once, that is, within 2 to 3 minutes after vein occlusion or, as is more often the case, the maximum is not reached until after 10 to 30 minutes. In table 1, experiment 5 illustrates this point. With the aortic pressure essentially constant at 109/90 mm. Hg the back flow rises from a control value of 1.54 cc. per minute to 5.4 cc. and 8.9 cc. per minute at 2 and 51 minutes respectively after clamping the sinus. Apparently it takes time for the coronary vascular communications to become maximally distended and as in experiment 6, once distended they do not at once revert to their former size.

In a few experiments analyses[5] of the blood coming from the peripheral coronary after occlusion of the cardiac venous system indicate that it is highly unsaturated and contains much less oxygen than does normal venous blood. In one experiment the backflow blood had an oxygen capacity of 19.9 cc. and a content of 4.0 cc., while blood taken simultaneously from the central coronary had an oxygen content of 17.4 cc. and that from the coronary sinus a content of 8.7 cc. In another experiment the peripheral coronary blood had an oxygen capacity of 17.4 cc., a content of 3.4 cc., while the central coronary and right ventricle had oxygen contents of 14.6 and 8.0 cc. respectively.

An attempt has been made to find the source of this extra backflow by clamping the other coronary arteries while the backflow was measured in the third coronary. The backflow in the descendens was then found to have its origin mainly in the left circumflex and this coronary, together

[5] We are greatly indebted to Dr. Edward Muntwyler of the Department of Biochemistry for these determinations.

with the right contributed nearly 100 per cent of the backflow after sinus compression (4 experiments). In experiment 4, table 1, the left circumflex accounts for 54 per cent and the right coronary 37 per cent of the additional backflow. Since these two coronaries were occluded separately and not simultaneously, the fact that the sum of the reduction in blood flow approximates 90 per cent does not necessarily mean that there was no other source of backflow. It is entirely possible that when one of the two vascular tubes which contributed to the backflow was occluded the flow through the other was increased. But the deduction can still be made that most of the backflow comes from the other two non-occluded coronaries.

It is established that coronary sinus occlusion, when added to occlusion of the left coronary, definitely elevates the peripheral flow and in some cases to such an extent that it might be sufficient, on the basis of volume flow alone, to maintain myocardial contractions, provided, of course, such retrograde blood reaches the myocardium under question when the peripheral coronary is not allowed to bleed externally. Accordingly, myographic records were taken from such muscle areas with combined central coronary and sinus occlusion, and in which the peripheral coronary was not allowed to bleed externally. In figure 1, *A*, *B*, *C*, are illustrations of such curves recorded, immediately, half a minute and 2 minutes, after ligation of the left descendens had been added to sinus ligation. In *B*, after 30 seconds the muscle is displaying signs of but feeble contractions and in *C* the myogram shows systolic extension.

Although such records, which have been obtained repeatedly, demonstrate that in the intact circulation sinus occlusion is of no value to a myocardium whose normal blood supply has been removed, it is possible that, when the peripheral coronary is allowed to bleed through its side branch against zero resistance (atmospheric pressure), that is, the condition under which backflows were actually measured, myograms might exhibit maintained contractions. Such curves, figure 1, *D*, *E*, *F*, are entirely similar to those in *A*, *B*, *C* and show no evidence of maintenance of systolic contraction. In *E*, taken 3 minutes after coronary blockage, the myocardium is contracting slightly, but in *F*, taken 2 minutes later, there is complete inversion of the myogram. Since central coronary occlusion alone causes failure of contraction to appear within approximately 2 minutes it is evident that sinus occlusion is of no material benefit to such hearts.

From such experiments we have reached the conclusion that the acute occlusion of a major portion of the venous drainage system of the heart does not aid the coronary circulation. The best evidence for this viewpoint is that when occlusion of the left coronary is added to sinus blockage such muscular areas fail to contract in approximately the same length

of time (2 min.) as do those in which the central coronary alone has been acutely closed. It makes no difference whether the central coronary is simply closed or allowed to bleed through a side branch; systolic shortening does not persist.

The question arises as to why this is so. Presumably after sinus or vein ligation the additional peripheral blood flow has its origin in the non-occluded coronaries (since occlusion of them essentially restores the back-

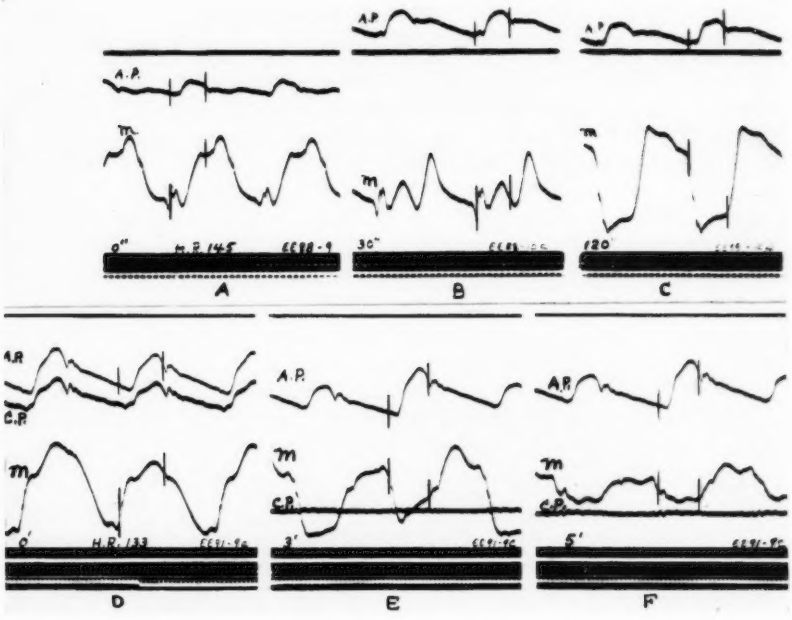

Fig. 1, A, B, C. Segments from records showing progressive failure of contraction after ligation of ramus descendens anterior and coronary sinus (peripheral coronary closed). *D*, *E*, *F*, segments taken under similar conditions but in which the peripheral coronary was allowed to bleed. *A.P.* = aortic pressure. *C.P.* = coronary pressure. *M* = myogram. Vertical intercepts = duration of systole. Time = 0.02 sec.

flow to normal figures) and is then routed in retrograde fashion from the cardiac venous system through a second set of capillaries, those of the occluded coronary (since the retrograde blood contains less oxygen than does that in the coronary sinus). As we see it the possible explanations why such blood does not support contractions are 1, although when the venous system is occluded, the pressure in it may rise appreciably, there is no anatomical circuit to force its blood through the capillary bed of the

occluded coronary artery, that is, the situation is analogous to trying to force blood through a tube closed at both ends; 2, an adequate circuit may be present, but it is largely on the venous side (through the large veins and Thebesians into the heart chambers) and hence blood does not flow through the capillaries of the occluded coronary but rather through vessels whose walls are not adapted for oxygen diffusion; 3, with an adequate anatomical circuit existing to route blood through the capillary bed, as, for example, when the peripheral coronary is allowed to bleed, the effective pressure for driving such blood in sufficient volume through the capillaries in retrograde manner is too low; 4, since the retrograde flow in the open peripheral vessel is almost completely unsaturated with respect to oxygen, the possibility exists that not enough oxygen is available even if the volume flow per se is sufficient. Although each of these factors is believed to be partly responsible for failure of contraction the relative importance of each cannot at present be evaluated.

However, when the peripheral coronary is allowed to bleed externally, and the coronary sinus is occluded, dynamic conditions should be set up which are favorable for adequate nourishment of the myocardium fed normally by the occluded coronary. Here there can be no question of the existence of an adequate and open anatomical circuit, for all possible sources of retrograde blood; Thebesians, great veins, arterioluminals and intercoronary anastomoses are operating against zero resistance at the cannula tip. Since, however, if a coronary alone is centrally occluded and allowed to bleed a maximum backflow of only 1.0 cc. per minute occurs, the only advantage of venous ligation to the heart is the blood under considerable pressure in the coronary sinus. Despite this addition, such myocardial areas do not exhibit any particular prolongation of time for maintenance of systolic contraction after arterial and sinus occlusion than do those in which the peripheral coronary has not been allowed to bleed.

This brings us to the possibility that the pressure difference between the source of the retrograde blood and the atmosphere is not sufficient to force through a sufficient minute volume of blood (with low oxygen content) to maintain contractions. Accordingly, to determine whether such areas could be made to contract, homologous arterial blood from other dogs was forced into the coronary sinus through a special cannula at a constant high pressure of 180 mm. Hg, and at a rate of approximately 150 cc./min., for 3 minutes, while the central end of the left descendens was occluded and allowed to bleed. Despite this addition of a seemingly adequate pressure difference and oxygen minute volume, the myocardium showed no evidence of maintenance of contraction while the peripheral coronary was either open or closed. The backflow (dark venous blood) increased from 3.5 cc. to approximately 15.0 cc./n in., table 1, experi-

ment 8. Although the area did not contract, arterial blood entered the vascular system as evidenced by the fact that the aortic pressure rose from 88/62 to 125/70 mm. Hg and the heart increased greatly in size. It is obvious that most of the infused blood entered the circulation either through the right heart or through the venous side of the capillary bed of the left coronary and hence could be of no great value to the myocardial area under study.

CONCLUSIONS

It has been established after acute coronary sinus ligation that:

1. The peripheral coronary backflow is markedly elevated in the ramus descendens anterior (up to 39 cc. per min.), but not in the right coronary.

2. The maximum retrograde flow is reached in from 10 to 30 minutes and following sinus release does not immediately return to the control backflow figure (maximum of approximately 1.0 cc. per min.).

3. The blood is highly unsaturated, containing only 3 to 4 volumes per cent oxygen.

4. Such a volume of blood with its low oxygen content is not sufficient to prevent failure of contraction when central occlusion of a coronary ramus is added to sinus ligation and hence is of no material value to the potentially infarcted myocardial area.

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THE IMMEDIATE EFFECTS OF THE OCCLUSION OF THE CORONARY VEINS ON THE DYNAMICS OF THE CORONARY CIRCULATION[1]

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In a previous paper (2) it was reported that acute occlusion of the coronary sinus in the anesthetized dog greatly augments the peripheral backflow of blood from a centrally occluded left coronary ramus. Indeed, with good blood pressures prevailing the magnitude of such backflows (up to 39 cc./min.) at times approaches the volume of blood which might be expected to flow into the central non-occluded coronary vessel. However, such backflow blood contains less oxygen than does that from the sinus and is of no material value to the potentially infarcted area as evidenced by the fact that myograms from such myocardial regions show systolic extension within 1 to 2 minutes after the coronary feeding that zone is occluded.

Although such experiments leave no room for doubt that coronary sinus occlusion is not immediately beneficial to the coronary circulation we became greatly interested, in view of the augmentation of peripheral coronary backflows, as to exactly what happened dynamically in the coronary vascular bed. Accordingly, we have studied the changes in cardiac venous pressure, peripheral coronary pressure and the coronary inflow after partial coronary venous occlusion.

The experimental set-up was similar to that previously described for measuring peripheral coronary backflows after venous occlusion (2). The changes in venous pressure in the heart were also optically recorded either by cannulation of the sinus or a vein. For recording the pressure in the coronary sinus a special cannula was devised (fig. 1) which was inserted directly through the right auricular appendage into the sinus where its tip was tied in place. When plunger tube, *F*, is in the position indicated in the diagram, blood flows from the coronary sinus through tube, *D*, and out through aperture, *E*, into the right auricle. By pushing

[1] Preliminary reports of this work have been presented at the American Physiological Society meeting in Baltimore, April 1, 1938 and before the Society for Experimental Biology and Medicine, Cleveland, Ohio, April 7, 1938.

plunger, *F*, beyond aperture, *E*, the drainage of venous blood into the sinus is blocked. Tube *C*, *B* is connected to the Gregg type (2, 3) of optical manometer so that the intrasinus pressure can be recorded with or without blockage of the sinus outflow. At other times venous pressures have been recorded by inserting a cannula connected to an optical manometer directly into a side branch of the great cardiac vein of the left heart.

The effect on coronary inflow into the left heart was studied by the method of differential pressure curves previously described (4). In brief, this method depends on determining the pressure difference between the aorta and the peripheral end of a coronary artery at various stages of a cardiac cycle, when flow in the coronary vessel is prevented by temporary and permanent clamping of the vessel central to the point of measurement. The height of such a curve (differential pressure) at any point gives velocity of flow and the area under any portion gives volume flow.

RECORDED RESULTS. *General observations.* Shortly after complete occlusion of the coronary sinus a number of events occur which are macro-

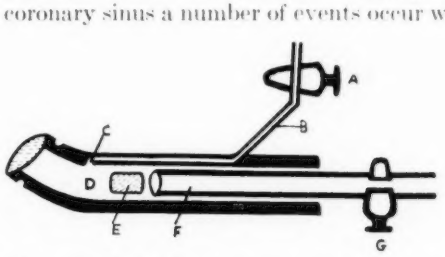

Fig. 1. Cannula for recording intrasinus pressure with coronary sinus open or closed. *A*, *B*, *C*, tube for leading intrasinus pressure to optical manometer. *E*, lateral opening from coronary sinus into auricle. *F*, tube for blocking sinus flow into auricle through *E*.

scopically observable. The heart rate immediately becomes slower by varying amounts. The aortic blood pressure decreases considerably (10 20 mm. Hg mean blood pressure). Within a few beats the heart becomes very dark in color, the veins stand out on the epicardial surface, small petechial hemorrhages appear and the diastolic size of the heart increases greatly. These changes are largely limited to the left ventricle while the right ventricle exhibits little, if any, distention, and remains pink in color with no visible evidence of venous congestion. These changes are the rule, but at times the blood pressure and heart rate are not decreased.

The peripheral coronary pressure curve. The effect of coronary sinus ligation on the peripheral pressure in the ramus descendens anterior is illustrated in figure 2, *A*, *B*, *C*. In *A*, before sinus occlusion, the aortic pressure is 74/46 mm. Hg, P.C.P. is 19/10 mm. Hg, and the heart rate, 110/min. In record *B*, taken 10 minutes after venous ligation, and with

the same heart rate and blood pressure prevailing, the P.C.P. rises to 65/20 mm. Hg. In *C*, 6 minutes after release of the sinus the peripheral pulse has returned essentially to normal values. (Coincident with this rise in peripheral pulse the peripheral flow increased from a normal of 0.4 cc./min. in *A* to 8.0 cc./min. in *B* and returned to 0.42 cc./min. in *C*.)

Comparison of the contour and time relations of the P.C.P. before and after sinus occlusion is also set forth in figure 2. In control *A* the P.C.P. curve starts to rise at *a*, very late in the isometric contraction phase and continues upward rapidly at first and then more slowly reaching its highest point just before the end of systole at *b*. Most of the rise occurs after the aortic valves are open. The curve now declines, reaching its diastolic level late in diastole. This agrees with the normal P.C.P. curves pre-

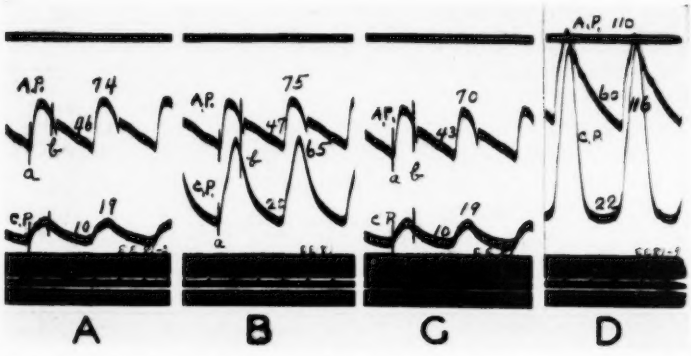

Fig. 2. Sections of records showing effect of sinus blockage upon peripheral coronary pulse in ramus descendens anterior. *A*, *C*, records taken before and after sinus occlusion in *B*. *D*, record in which P.C.P. exceeds aortic pressure. *A*, *P*, aortic pressure. *C*, *P*, peripheral coronary pressure. Time, 1/5 sec.

viously reported (5). Following sinus ligation in *B*, the P.C.P. undergoes no change save in magnitude, i.e., the rise still occurs almost entirely after the opening of the aortic valves. This similarity between the contour and time relations of the P.C.P. before and after sinus ligation has held in all our experiments. However, in some experiments (generally those with higher aortic blood pressure) a good portion of the rise of both venous and P.C.P. curves occurred earlier, i.e., during the isometric contraction period.

Although in all records following sinus occlusion the peripheral coronary systolic and diastolic pressures are greatly increased as in figure 2, *B* (cf. also fig. 3, *B*, fig. 4, *H*, *I*, *J*), the systolic maximum, ranging between aortic systolic and diastolic pressures, can go much higher. In some experiments the systolic peak equals or exceeds the simultaneously recorded

systolic value in the aorta. An example of such a relationship is set forth in figure 2, *D* in which the peripheral coronary systolic pressure is 116 mm. Hg, as compared with 110 mm. Hg for aortic systolic. Pressures, however, which exceed aortic systolic are not a common finding, whereas values approaching aortic systolic are characteristic.

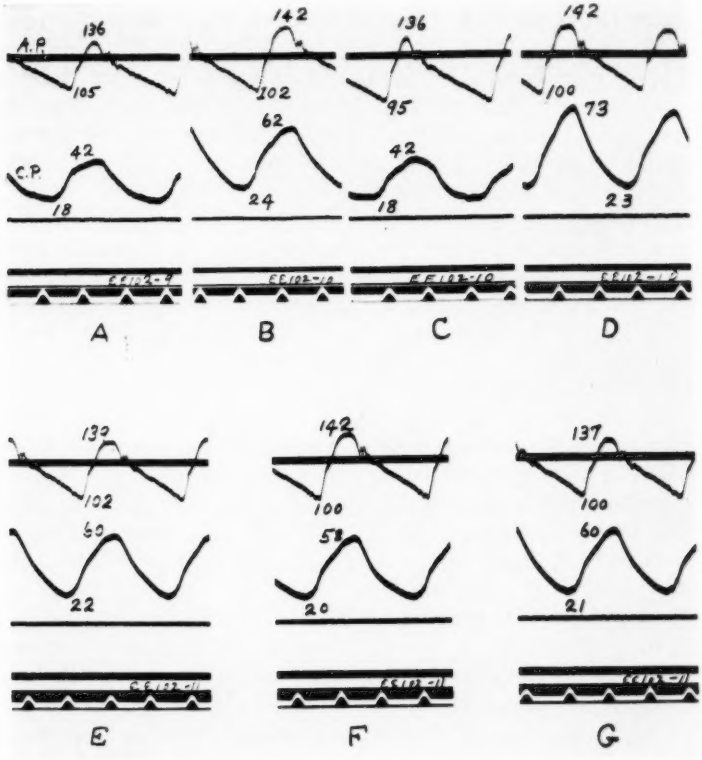

Fig. 3. Reproductions of records illustrating effect of occlusion of right coronary and left circumflex upon the P.C.P. in ramus descendens anterior after sinus ligation. *A*, normal P.C.P. *B*, *D*, control P.C.P. curves with coronary sinus occluded and taken before and after left circumflex occlusion in *C*. *E*, *G*, similar controls before and after right coronary blockage in *F*. Letters and time interval same as in figure 2.

As a rule it is a matter of some minutes after sinus occlusion before the peripheral coronary pressure attains its maximum. Thus in a typical experiment the aortic pressure was 105/88 mm. Hg and the peripheral pressure, 29/11 mm. Hg. After 1 and 21 minutes' occlusion of the sinus

the peripheral pulse was 56/30 and 87/37 mm. Hg respectively. After 51 minutes of clamping a peak value of 100/47 mm. Hg was reached, while the aortic pressure was 112/94 mm. Hg. Coincident with the progressive rise in P.C.P. the coronary backflow increased from 0.98 cc. per minute to 9.0 cc. per minute. In this experiment (contrary to most) the rise of the P.C.P. in both normal and after sinus occlusion occurred largely during the isometric contraction period. We have only occasionally observed this phenomenon.

Having established that after sinus occlusion the peripheral pulse in the left coronary is greatly elevated, our attention was next turned to the source of this extra pressure head. In figure 3 is presented evidence that the augmentation of the peripheral pulse has its origin in the other two non-occluded coronary arteries. In *A*, taken before sinus ligation the P.C.P. in the ramus descendens anterior is 42/18 mm. Hg. In *B*, after sinus occlusion for 5 minutes, the peripheral pulse rises to 62/24 mm. Hg. In *C*, taken approximately 30 heart beats after the left circumflex was also occluded the peripheral pulse drops to the control value of 42/18 mm. Hg in *A*. In *D* the left circumflex has been released for 1 minute. *E*, *G* are the control records taken before and after right coronary occlusion in *F* is added to sinus occlusion. The reduction in peripheral pressure from 60/22 mm. Hg to 58/20 mm. Hg is just perceptible. In this, as in a number of other experiments in which the source of the extra pressure head in the left descendens has been determined, it has been largely accounted for by the other non-occluded coronary arteries, especially the left circumflex. However, in some of these experiments a somewhat larger portion of the venous pressure increment (up to 30 per cent) *has its* origin in the right coronary.

The cardiac venous pressure curves. It would seem likely that the mechanical constriction of one of the major venous drainage systems of the heart should create a condition of increased pressure in the cardiac veins. Hence, pressure curves were recorded from the venous system after coronary sinus or cardiac venous ligation. Pressures from the sinus were taken through the special cannula already described in figure 1. Curves from different parts of the venous sytem in different experiments and with varying degrees of venous constriction, together with the pressure pulses from the aorta and/or left coronary are illustrated in figure 4. In *A*, with the sinus occluded by the instrument of figure 1, the sinus pressure is 135/24 mm. Hg, while that in the aorta is 90/50 mm. Hg. In *B* and *C* the sinus was occluded by means of a clamp and the venous pressure recorded through a branch of the great cardiac vein. In *B*, the venous pressure, 68/30 mm. Hg, is considerably less than aortic, 105/70, while in *C* it becomes greater than aortic systolic (157/28 mm. Hg, as

compared to 133/80 mm. Hg). It has been our experience that after sinus occlusion the pressure in the venous system is generally less than that

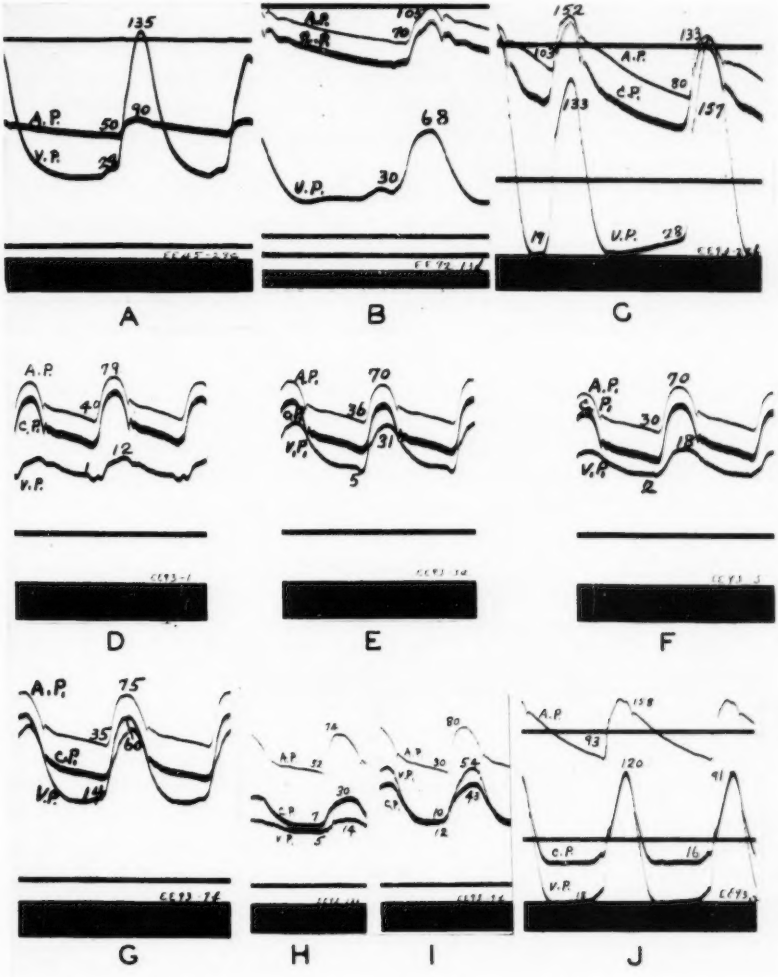

Fig. 4. Comparison of pressure curves from aorta (*A. P.*), peripheral or central coronary (*C. P.*), coronary sinus or great cardiac vein (*V. P.*) under different dynamic conditions and with different degrees of venous constriction. Discussion in text.

in the aorta or central coronary, although, as illustrated above, it can at times exceed the aortic systolic maximum.

D, E, F, G of figure 4 contrast the effects on venous pressure of occluding the great cardiac vein and the coronary sinus in the same experiment, the venous pressure being recorded through a side branch of the great cardiac vein. In *D* the pressure in the vein without occlusion is 12/1 mm. Hg. In *E* the great cardiac vein was occluded just under the auricle and the venous pressure rose to 31/5 mm. Hg. In *F* the vein was released and the pressure dropped toward the control values in *D*. The sinus was then occluded and the venous pressure rose to 60/14 mm. Hg in *G*, or to a value approaching aortic systolic.

Such records which have been obtained in 3 experiments indicate that vein occlusion is by no means as potent a factor in raising the pressure in that vein as is occlusion of the sinus.

As a rule, the contour and time relations of such venous pressure curves parallel quite closely those in the peripheral coronary artery, while the actual pressures exceed the P.C.P. values. In figure 4, *H, I, J*, are records of pressures recorded simultaneously from the aorta, peripheral coronary (left descendens) and the great cardiac vein under different dynamic conditions. Casual inspection of these curves indicates that the coronary and venous pressures rise and fall simultaneously and as in *J* become superimposable. In *H*, before the great cardiac vein was occluded peripherally, the pressure in it was 14/5 mm. Hg, while the P.C.P. was 30/7 mm. Hg. The coronary sinus was then occluded (*I*) and the venous pressure rose to exceed that in the peripheral coronary 54/12 mm. Hg, as compared with 43/10 mm. Hg. In *J*, taken at a higher blood pressure (aortic compression) and after sinus occlusion, the venous pressure, 120/18 mm. Hg, also exceeded that in the peripheral coronary, 91/16 mm. Hg.

Phasic coronary blood flow after sinus ligation. In addition to recording the peripheral coronary pressure after cardiac vein occlusion, we have also determined its effect on the maximum systolic resistance to coronary inflow. This was done by intermittent clamping of a branch of the left coronary for a portion of systole and at various systolic times in different cycles until a time of clamping was found during which the peripheral pressure neither rose nor fell during systolic clamping. The procedure has been described previously (4). This was taken as the measure of maximum systolic resistance to inflow. Such curves proved that the systolic resistance to coronary inflow is always elevated after sinus ligation but the magnitude of the change is variable, the figures ranging from pressures considerably less than aortic systolic to those which exceed it. Examination of all our curves indicates that the usual result is an elevation of the peripheral pressure to values approaching aortic systolic.

Since, following cardiac vein ligation, the resistance to coronary outflow is elevated (by increase of venous pressure) and the peripheral coronary and systolic holding pressures are also augmented, it would be predicted that this combination would result in a net decrease in coronary inflow.

In figure 5 is shown the effect of sinus occlusion upon arterial inflow into the ramus descendens anterior. The blood flow was determined by our

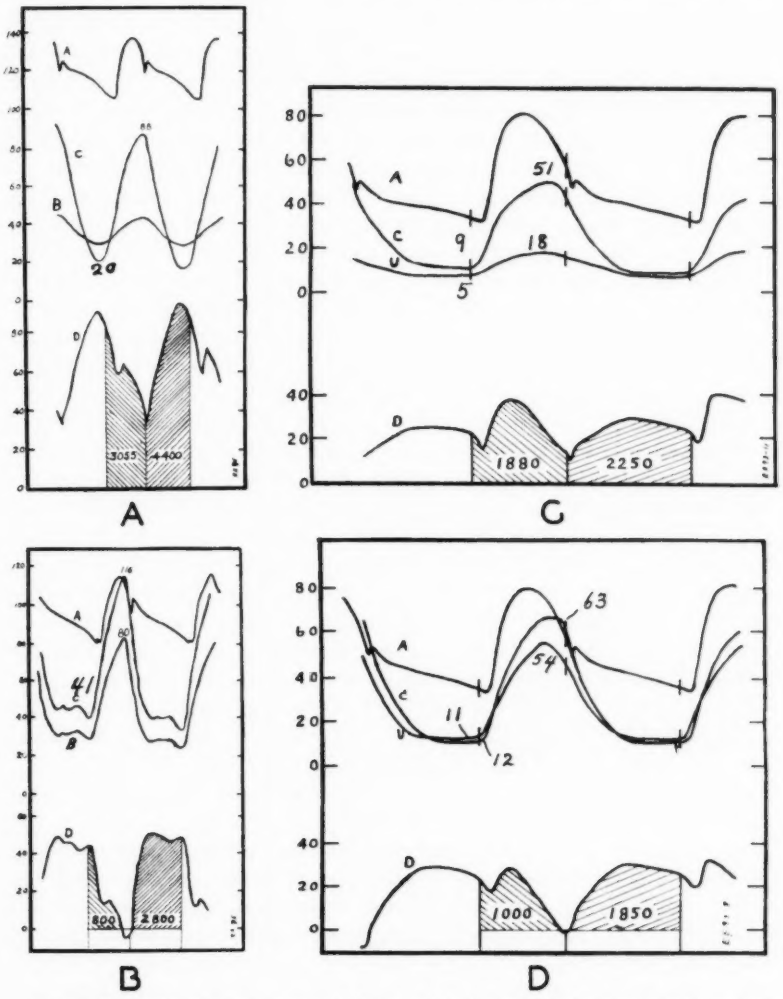

Fig. 5. Four charts reconstructed from original records showing phasic volume flow and velocity in intramural vessels before (charts *A*, *C*) and after (charts *B*, *D*) coronary sinus ligation. *A*, aortic pressure; *B*, peripheral coronary pressure; *C*, same reconstructed with true ordinate values; *D*, velocity curve; *V*, intravenous pressure; shaded areas, volume flow. Ordinates, mm. Hg. Abscissa, time.

standard procedure of differential pressure curves (4). To conserve space, the original records are not included, but only the reconstructed drawings

shown in figure 5. In *A*, with the sinus unoccluded, the systolic and diastolic flows, empirically expressed, are 3055 and 4400 sq. mm. area. This volume relationship, as well as the relative velocities of systolic and diastolic flow, are similar to those previously published as normal inflow curves (4). The curves from which *B* was reconstructed were recorded 15 minutes later in the same experiment and 10 minutes after coronary sinus ligation. The heart rate is the same but the aortic pressure is somewhat lower. Despite the lower blood pressure which in previous publications (6) was discovered to lower both systolic and diastolic peripheral resistance, it is found here that the systolic coronary resistance rises from 88 mm. Hg in *A* to a value equal to aortic systolic of 116 mm. Hg, while the diastolic is moderately elevated from 20 mm. Hg to 41 mm. Hg. Since the time relations and contour of the peripheral pulse are essentially unaltered it follows that both the systolic and diastolic flows are significantly reduced as the result of venous occlusion, the former to 800 sq. mm. area, and the latter to 2800 sq. mm. area. This is due to the higher peripheral pulse and in part to the lower aortic blood pressure.

Comparison of flow curves in *C* and *D* (in which dynamic conditions are not as good as in the above) also show systolic and diastolic flow reductions, but of a lesser degree. In the original records the venous pressure was also simultaneously recorded from a side branch of the great cardiac vein and rose from 18/5 mm. Hg in *A* to 54/12 mm. Hg in *B* after sinus occlusion. With the heart rate and blood pressure constant, the peripheral coronary diastolic pressure rises to 11 mm. Hg from a control value of 9 mm. Hg, while the systolic holding pressure is elevated from 51 to 63 mm. Hg. As a result of this the systolic flow is reduced by 47 per cent and the diastolic by 18 per cent.

Following cardiac vein ligation the reduction in coronary inflow may therefore be large or small, most of the decrease occurring during systole. Our experience has been that the usual reduction is nearer the larger figure.

Discussion. When the coronary sinus or a portion of the cardiac venous system is abruptly occluded the following dynamic changes occur: 1, the diastolic size of the heart is increased; 2, the left ventricle shows marked venous congestion in contrast to the right, which remains relatively pink in color; 3, the intravenous pressure rises from quite low values approximating 10/2 mm. Hg to figures which during systole approach, equal, or exceed the aortic systolic pressure, and during diastole equal 20–40 mm. Hg; 4, the peripheral coronary pressure is elevated in like fashion, the systolic figure hovering just below or above aortic systolic; 5, the arterial inflow into the left coronary is significantly reduced, and 6, the retrograde blood flow from the anterior descendens rises from a control maximum of 1 cc. per minute to as high as 40 cc. per minute (2).

The probable significance of these changes can best be approached

through a schematic diagram, figure 6, *A*. In the normal heart all three major coronaries rami are joined on the arterial side by connections *a*, *b*. Each artery has arterial and venous communications with ventricles and auricles by channels, *b*, *d*, *e*, *f*, after which all three vascular beds are joined to form the coronary sinus.

Presumably most of the blood normally reaching the coronary sinus has its origin in the coronary arteries (7) and enters the sinus during systole and diastole (8). However, it may also be predicted that a sizable blood flow also occurs from either ventricular cavity to veins via Thebesian circuits. This is so because, when the probable pressure relationship exist-

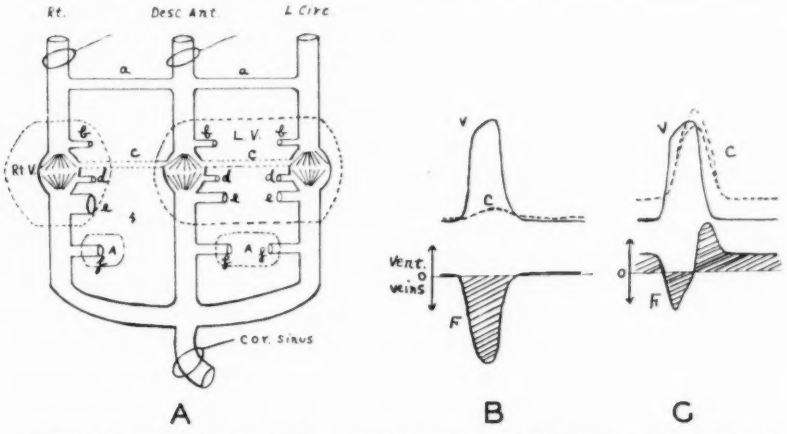

Fig. 6. Chart *A*, schematic diagram illustrating probable anatomical circuits of the coronary vessels. *Rt. V*, right ventricular cavity; *L.V.*, left ventricular cavity; *A*, auricles. *B*, *C*, charts showing probable direction and magnitude of blood flow between ventricles and coronary veins before (chart *B*) and after (chart *C*) coronary sinus ligation. *V*, ventricular pressure; *C*, pressure in coronary vein; *F*, blood flow. Discussion in text.

ing between ventricle and venous system (curves *V* and *C*, fig. 6, *B*) are graphed, using the same ordinate scale, the result is a marked systolic blood flow (curve *F*) from ventricle to veins. This should occur especially in the left ventricle, since its pressure is much greater than that in the right ventricle.

After increased cardiac venous resistance of sinus origin an additional mean volume of blood is trapped in the venous system of the heart. The pressure created directly or indirectly by the left ventricle via vascular channels is now transmitted better to the interior of the large veins, for the whole vascular bed is greatly distended, and as a result the pressure within them rises significantly (for example, as in fig. 4, *A*, *C*). This

additional pressure and blood must have its origin largely by direct feed of the coronary arteries through the capillary bed into the venous system. It would now be expected that either very little blood would enter the venous system from the ventricles or that actually blood would now flow from the venous system into these cavities especially during diastole, for the pressure relations between the possible anatomical feed system (*d*, *e*, *f*, fig. 6, *A*) from auricles and ventricles into the venous system, have now been reversed from the normal. This relationship is set forth graphically (fig. 6, *C*). Apparently the capacity for such retrograde flow is greater in the right heart than in the left for it has been repeatedly observed that there is very little visible evidence of venous congestion in the right heart following venous ligation. This would be expected since the intraventricular pressure and intravascular resistance in the right ventricle are much less than the respective pressures in the left ventricle (9).

If now the proximal end of a left coronary artery is also abruptly occluded conditions for retrograde flow from veins into the left ventricular cavity are further enhanced via channels, *d*, *e*, figure 6, *A*, of the peripheral vascular bed of the occluded coronary and are made possible through *b*, for both the systolic and diastolic peripheral pressures are greatly reduced and are generally less than the simultaneously recorded pressures on the venous side (figs. 2, 3, 4). Consequently as a result of occlusion of the sinus and a left coronary ramus such dynamic conditions are created that there should be a sizable blood flow from the non-occluded coronaries through their capillary beds into the large veins and then by Thebesians and arterio-luminal vessels into the ventricular lumen. If now the peripheral coronary is opened so that the venous pressure is operating against essentially zero resistance at the cannula tip a goodly portion of this blood could be diverted from the venous system through the capillary bed into the cut artery. The blood flow from the peripheral coronary will be determined largely by the rise in venous pressure, which in turn is governed by the resultant of the capacity of the Thebesian system for draining blood into the heart cavities versus the mean volume of blood entering the venous system from the non-occluded coronaries.

The marked increases in systolic and diastolic peripheral coronary pressures (figs. 2, 3, 4) after sinus occlusion, as compared with normal values are also to be attributed largely to the increased distention of the arterial bed by blood coming from the venous system. This blood and pressure must have its ultimate origin in the other non-occluded coronaries, for both are reduced to control values by occlusion of the other coronaries (fig. 4) (2).

From such observations it is to be expected that the coronary inflow into the left heart will be considerably reduced (fig. 5). Since, however, relatively large residual coronary inflows still remain and the myocardium

still contracts, whereas the area fails to contract when the pressure gradients are reversed in the same set of tubes (fig. 6, *C*) by a combination of coronary sinus and coronary artery blockage and massive infusion of arterial blood into the sinus at high pressure (2), the conclusion seems justified that, in a coronary with both ends blocked, potent drainage channels still remain and that these are largely venous, that is, they are similar to *d*, *e* and *f* of figure 6, *A*, and hence are of no material value to the myocardium, despite the large backflows of blood which occur when the peripheral coronary is opened. Apparently these Thebesian channels are more useful in the right ventricle than in the left. Although no inflow curves have been obtained in the former following sinus ligation, the inflow can scarcely be reduced, since the peripheral pulse in the right coronary is not elevated by such a procedure (unpublished experiments).

A point of considerable interest in these experiments on venous occlusion is the observation that the pressure during systole in the venous system and in the peripheral left coronary can exceed that in the aorta. This relationship is not the rule, but it has been repeatedly observed in curves exhibiting no artefacts and represents to us a true picture of what such pressure relations can be. It is, of course, to be expected that the degree of filling of these vessels on arterial and venous sides determines in part the pressures created in them by a compressing force, the surrounding myocardium. When the sinus is clamped the filling is obviously greater and one would expect the intra-vessel pressure to approach at least that in the ventricle. Physical explanations can be advanced to explain how the intra-vessel pressure (and hence intramural pressure) can exceed intraventricular pressure. However, our experimental information is too meager and the situation too complex to warrant discussion at present.

SUMMARY

The phasic changes in venous pressure, peripheral coronary pressure and arterial inflow in the heart have been studied by optical methods before and after acute cardiac venous ligation (coronary sinus or great cardiac vein).

The intravenous pressure rises from control values of say 10/2 mm. Hg to figures which during systole approach or exceed the aortic systolic and whose diastolic value rises to 20–40 mm. Hg.

The peripheral coronary pressure, simultaneously recorded, is elevated in the left coronary in like degree and has a contour and time relation similar to the venous pressure, although its ordinate values are generally slightly less. Probable explanations for these changes in venous and peripheral coronary pressure are advanced.

The arterial inflow into the left coronary is reduced considerably, while that into the right coronary is presumably not affected. Since, however,

the left coronary inflow does not approach zero and the myocardium still contracts it is believed that there are potent drainage channels still remaining. These are presumably venous, since if a left coronary ramus is now occluded the myocardium fails to contract even if the peripheral coronary is allowed to bleed.

Since it has been found that acute cardiac venous ligation does not prevent failure of contraction in a myocardial area whose coronary has been ligated and does reduce materially the left coronary arterial inflow, such a procedure cannot be regarded as a method of choice for encouraging the blood supply to such a potentially infarcted area.

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RESPIRATORY AND CIRCULATORY ADJUSTMENTS TO THE ERECT POSTURE

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That there is in man a marked decrease in the CO_2 content of the alveolar air upon assuming the erect posture has been established by the work of Liljestrand and Wollin (1913), Higgins (1914), Turner (1927) and others. Main (1937) has not only confirmed the observations of the earlier workers but has critically considered and evaluated the various theories advanced by different investigators in explanation of this phenomenon. He finally concludes that "the diminished alveolar CO_2 upon standing is .... due to pulmonary over-ventilation with resulting alkalemia." A large volume of data on the respiratory exchanges in various postures accumulated by one of us (F. A. H.) in other investigations made such an assumption seem improbable. Therefore, further investigation as to the cause of this phenomenon seemed desirable and the experiments reported here were undertaken with this object in view.

METHOD. In the first series of experiments the complete respiratory exchanges were determined during a reclining period and an immediately succeeding period of standing. Six adult male subjects were used in these experiments. The typical procedure was as follows. The subject lay on a comfortable bed for from ten to fifteen minutes to attain a steady state. During the next five to ten minutes the expired air was collected in a spirometer of the chain compensated type. At the end of this period alveolar air was collected by the method of Carpenter and Lee (1933). The subject then stood up and was at once connected to a second spirometer. The expired air was again collected for a period of from five to ten minutes at the end of which another collection of alveolar air was made. Expiratory and inspiratory samples were taken each time. The results reported are an average of these two values. All samples of expired and of alveolar air were analyzed by means of the Haldane air analysis apparatus. Some experiments were performed in the post-absorptive condition and others after the subject's usual breakfast. It was not observed that the breakfast had any pertinent effect. The respiratory rate was usually recorded by means of a pneumograph.

RESULTS. The results obtained in this first series of experiments are

summarized in table 1. It will be noted that all six subjects showed a marked decrease in the alveolar CO_2 upon assuming the erect posture. The smallest drop was 7.8 per cent with the subject D. E. R. while two of the subjects (B. A. R. and B. U. R.) showed decreases of more than 12 per cent. In all of our experiments this drop invariably occurred and therefore in this respect our results are in complete agreement with the work of earlier investigators.

The respiratory quotients reported in table 1 are of particular significance. With the first four subjects listed, upon whom repeated tests were run, there was an appreciable drop in the quotient upon assuming the erect posture. With the subjects B. U. R. and D. E. R. there was no significant change. In the entire series of twenty-one experiments there were only three in which the quotient was not lower during the standing

TABLE 1

Data on respiratory exchanges during reclining and standing

SUBJECT	NUMBER OF TESTS	ALVEOLAR CO_2		R.Q.		O_2 CONSUMPTION		VENT. VOL.		VEN. EQ.*	
		Reclining	Standing	Reclining	Standing	Reclining	Standing	Reclining	Standing	Reclining	Standing
						cc./min.	*cc./min.*	*l./min.*	*l./min.*		
R. H.	4	5.80	5.19	0.82	0.79	286	256	5.00	6.20	1.93	2.16
J. K. W.	5	5.78	5.31	0.91	0.83	252	276	5.91	6.89	2.34	2.51
F. H.	5	6.60	5.98	0.85	0.76	270	292	5.23	4.84	1.86	1.65
B. A. R.	5	6.06	5.43	0.83	0.79	303	265	4.31	5.48	1.62	1.81
B. U. R.	1	5.76	5.02	0.83	0.84	264	216	4.73	7.04	2.19	2.66
D. E. R.	1	5.89	5.43	0.85	0.84	261	258	6.05	6.03	2.31	2.34

* The ventilation equivalent is equal to the liters of air breathed per 100 cc. of oxygen absorbed.

test than during reclining. It is therefore evident that there was no over-ventilation in the sense that CO_2 was being eliminated more rapidly than it was being produced. These data are incompatible with the theory that the drop in alveolar CO_2 which occurs on standing is associated with the depletion of CO_2 stores of the body by over-ventilation.

The data on ventilation volume have a direct bearing on this matter. Table 1 shows that the ventilation volume on standing remained practically unchanged in the one experiment on D. E. R., consistently decreased in each of the five experiments on F. H., but invariably rose with the remaining four subjects. The ventilation equivalents shown in the final column of table 1 are perhaps even more significant than the ventilation volumes, since they show the number of liters of air breathed per 100 cc. of O_2 absorbed. Upon assuming the erect posture F. H. showed an 11 per cent drop in this figure, D. E. R. showed no significant change, and the

other four subjects showed rises ranging from 7 to 21 per cent. Thus it is evident that F. H. showed a drop in alveolar CO_2 in spite of an 11 per cent drop in the quantity of air breathed per 100 cc. of O_2 absorbed. Furthermore, since the four subjects that showed increases in the ventilation equivalent failed to show any increased CO_2 output in proportion to the O_2 absorption, as is evidenced by the failure of the quotient to rise, it cannot be argued that the drop in alveolar CO_2 occurring in these subjects was in any sense associated with over-ventilation. These results justify the conclusion that the drop in alveolar CO_2 which occurs upon assuming the erect posture is independent of any change in pulmonary ventilation and suggests that other factors, perhaps circulatory adjustments and changes in lung volume, may be causative factors in the production of this phenomenon.

Oxygen debt. A study of the data on O_2 consumption recorded in table 1 reveals the rather startling fact that in four of the six subjects there was actually less O_2 consumed per minute during the brief period of standing than during the preceding period of reclining. In view of the fact that the muscular exertion of standing must of necessity, increase the energy utilization of the body, these results suggested that an O_2 debt was accumulated during the period of standing. Accordingly experiments were designed to test the truth of this hypothesis. In these experiments the respiratory exchanges were followed during a second reclining period immediately following the standing period. In five such experiments carried out on four different subjects, who stood for a period of from five to eleven minutes, O_2 debts were invariably observed. They ranged from 247 to 536 cc. of O_2. The prolongation of the standing period beyond five minutes in one subject was without effect on the magnitude of the O_2 debt.

To determine the length of time required to pay off this O_2 debt, other experiments were conducted in which the O_2 consumption during the recovery from standing was followed during consecutive periods of one, two, three, and four minutes. In five such experiments the O_2 consumption rose during the first minute of recovery to a value 30 to 170 per cent higher than the O_2 consumption during a period of reclining preceding the period of standing. The average increase was a little more than 100 per cent and in only one of the five subjects was the increase less than 90 per cent. The total O_2 debts observed in these experiments ranged from 396 to 700 cc. (average 530 cc.), and in all five subjects recovery was practically complete in three minutes or less.

The respiratory quotients in the reclining periods which preceded standing, averaged 0.82 (range 0.79 to 0.85). In all five subjects there was a significant drop in the quotient during the first minute of recovery. This drop averaged 0.09 (range 0.04 to 0.13) and in only one case was the drop less than this average. During the subsequent periods of recovery the

respiratory quotient rose until it reached a value approximately the same as that of the reclining period preceding standing. These results are in substantial agreement with those reported recently by McMichael (1937).

Blood gases. It has already been pointed out that there was no rise in the R. Q. during the period of standing. Indeed with most of the subjects there was a significant drop. It therefore follows that there must have been a retention of CO_2 during this period of a magnitude as great or greater than the O_2 debt which accumulated. Since the imperfections of the human circulatory system are such as to result in a considerable degree of stagnation of the blood of the lower half of the body during quiet standing, it seemed likely that the bulk of this retained CO_2, and perhaps an appreciable proportion of the O_2 debt would be found in the blood of the lower limbs. Accordingly a series of experiments was carried out in which samples of venous blood were drawn from a foot and a hand, first,

TABLE 2

The changes in CO_2 and O_2 content of venous blood on standing after reclining

SUBJECT	FOOT		HAND	
	Δ CO_2 v.p.c. in plasma	Δ O_2 v.p.c. in red cells	Δ CO_2 v.p.c. in plasma	Δ O_2 v.p.c. in red cells
F. A. H.*	+2.4	−3.5	−2.8	+7.6
F. A. H.	+1.9	−4.7	−6.2	+14.1
V. I. N.	+5.1	−8.7	−1.7	−4.0
H. I. N.	+0.2	−2.4	−2.9	+1.2
M. A. U.	+1.5	−0.9	−0.5	−3.6

Note: In all experiments except the one marked * the foot was warmed to facilitate the drawing of blood.

after a reclining period of from fifteen to twenty minutes, and second, after a period of quiet standing of from five to ten minutes. Considerable difficulty was experienced in obtaining the blood samples from the feet during the reclining period since in this position there was a tendency for the blood to drain out of the large veins. It was therefore necessary to allow the foot from which the sample of blood was to be taken to hang over the edge of the bed upon which the subject lay. As an additional means of stimulating blood flow in most of the experiments the foot was warmed by immersing it for about five minutes in water at 45°C. In order that the conditions of the experiments should be uniform the foot from which the blood was drawn at the end of the standing period was also immersed in water at the same temperature. The results of five such experiments carried out on four different subjects are shown in table 2. It will be noted that in every case the CO_2 content of the blood taken from the foot increased and the O_2 content decreased during the period of

standing. The changes observed in the gaseous content of the blood drawn from the hand were, as a rule, in the opposite direction; that is at the end of the standing period the CO_2 content of the venous blood in the hand was always less, while the O_2 content was usually greater than before. These results are in agreement with the findings of Main (1937) who reported that the venous blood of the arm became more alkaline in the erect posture. Main, however, assumed that this change in the blood was the result of increased pulmonary ventilation, while in the light of our findings we feel that we may say with a fair degree of finality that it is rather due to a lack of complete circulatory adjustment to the change in posture. In the erect posture the bulk of the body (the head which is above the heart as well as the abdominal viscera and lower limbs which are below the heart) is at a circulatory disadvantage. This circulatory disadvantage would result in a slowing of the circulation rate which in some cases might even amount to a partial stagnation. From these considerations, therefore, we may assume that the bulk of the blood would behave like the samples taken from the feet, that is, the venous blood would show an increased CO_2 content and a decreased O_2 content.

On the other hand there seemed to be an improvement in the circulation through the arms and thorax. This view is supported by the changes in blood gases which occurred in the samples taken from the hands. The two subjects, V. I. N. and M. A. U., who showed a decrease in the O_2 content of blood taken from the hand, may have had some slight impairment of the circulation even in this region, although it is difficult to harmonize such an assumption with the decreased CO_2 content of the venous blood taken from the hand, which occurred in all subjects. This is presumably a reflection of the fall in arterial CO_2 which accompanies the lowered alveolar CO_2. This decreased arterial CO_2 is not reflected in the venous blood of the foot because of the stagnation of the circulation in this part of the body. We are therefore justified in concluding that the circulation through the arms and thorax is more efficient in the erect posture than that through the lower limbs and abdomen.

The assumption of a decreased circulation rate in the bulk of the body in the erect posture is in qualitative agreement with the observed O_2 debt and CO_2 retention. The quantitative agreement may be tested by calculation as follows: With the subject F. H., upon whom two determinations of blood gases were made, there was an average decrease of 4.1 volumes per cent in the O_2 content of the red cells of the venous blood of the foot after standing quietly for from five to ten minutes. This subject weighed about 70 kilos and would therefore have a blood cell volume of about 2800 cc. Assuming that two-thirds of this volume is at a circulatory disadvantage we may calculate that 1867 cc. have the same O_2 deficit as that observed in the blood of the feet. On this basis the total calcu-

lated O_2 deficit of the blood would be about 75 cc. The respiratory experiments on this subject showed that at the end of the period of standing he had accumulated an O_2 debt of nearly 400 cc. It should be pointed out, however, that warming the foot in the standing position and allowing the foot to hang over the edge of the bed in the reclining period are both procedures which would tend to decrease the expected differences in blood gas content. But even after making allowance for all of the possible sources of error it would still seem unlikely that the entire O_2 debt could be accounted for on the basis of the increased reduction of the hemoglobin in those parts of the body that are at a circulatory disadvantage, and we conclude, therefore, that a large proportion of the total O_2 debt is in the tissues themselves.

As might be expected, since plasma CO_2 is known nearly to parallel tissue fluid CO_2, the differences observed in the CO_2 content of blood drawn from the foot in the reclining and erect postures give much better quantitative agreement with the observed CO_2 retention. The average change in CO_2 content of the plasma for the entire group was 2.52 v.p.c., this being an increase in the lower part of the body and a decrease in the upper part. The average weight of the subjects was about 70 kilos and assuming that 70 per cent of this weight is body fluids, we calculate a total volume of 49 liters. As already pointed out we may assume that two-thirds of this total behaves like the blood from the foot while one-third behaves like the blood from the hand. From this we calculate that the total CO_2 retained was more than 400 cc., a figure of the same general magnitude as that found in the studies on respiratory exchanges.

It has already been pointed out that the respiratory quotients dropped sharply at the beginning of recovery and then rose slowly to approximately their initial values (see p. 459). This behavior of the R.Q. can be best explained by assuming that the retained CO_2 was given off during the recovery period at a slower rate than that at which the O_2 debt was paid. This is in agreement with the findings of McMichael (1937).

Lung volume. Another factor which seems to us of paramount importance has not been considered by previous workers. This is the change in the volume of the functional residual air which occurs upon assuming the erect posture. That there is a marked increase in the volume of the functional residual air upon changing to either a sitting or a standing position from a reclining posture has been demonstrated by Hurtado and Fray (1933), and by numerous earlier workers cited in their paper. We have verified the findings of these earlier workers by measuring the vital capacity, the complemental air and the supplemental air in both the reclining and standing positions on six normal adult male subjects. The results of these measurements are shown in table 3. It will be noted from this

table that the changes in vital capacity upon assuming the erect posture were almost negligible. In two of the subjects there was no change at all and in the other four a slight increase. The average for the entire group was an increase of a little more than four per cent. The complemental and the supplemental air both showed, however, profound changes when the subjects changed from the reclining to the standing position. The supplemental air invariably increased, the average increase for our six subjects being 519 cc. or 53.5 per cent. The change in complemental air was of a nearly equal volume but in the opposite direction. For our subjects it averaged 471 cc. (16 per cent). This effect is probably due to the influence of gravity in lowering the resting level of the diaphragm. If we assume that the residual air has a volume of about 1500 cc. the functional residual air would have changed from an average value of 2470 cc. in the reclining position to 2990 cc. in the erect position. If the level

TABLE 3

Data on the vital capacity and its subdivisions during reclining and standing

SUBJECT	VITAL CAPACITY		COMPLEMENTAL AIR		SUPPLEMENTAL AIR		TIDAL AIR	
	Reclining	Standing	Reclining	Standing	Reclining	Standing	Reclining	Standing
	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
R. E.	4,436	4,768	3,130	2,653	943	1,612	415	487
B. N.	4,250	4,519	2,591	2,197	995	1,472	705	891
M. S.	4,415	4,415	3,192	2,446	891	1,430	788	384
M. D.	5,514	5,514	3,233	2,819	1,575	1,949	767	767
S. N.	3,959	4,291	2,964	2,570	497	933	539	808
P. T.	4,083	4,333	2,591	2,187	912	1,534	601	622
Average.	4,443	4,640	2,950	2,479	969	1,488	636	660

of the alveolar CO_2 was six per cent in the reclining position, the drawing into the lungs of the more than 500 cc. of air that were added to the supplemental air upon standing erect, would have the effect of reducing this figure to a value of less than five per cent. However if this change in the volume of the functional residual air were the sole factor involved, the decreased alveolar CO_2 would be only temporary, for the giving off of only 30 cc. of CO_2 from the reserves of the body would be sufficient to bring the increased volume of functional residual air back to the composition of the original alveolar air. This would produce a negligible decrease in the CO_2 content of the body fluids and after equilibrium had been reëstablished the result would be a practically unchanged alveolar CO_2. At this point, however, the impaired circulation steps in and prevents the reëstablishment of equilibrium at the old level. The partial stagnation of blood in those portions of the body that are at a circulatory disadvantage in the

erect posture is equivalent to the withdrawal of a substantial quantity of blood from the pulmonary circulation. According to Grollman (1932) the cardiac output is unchanged in the erect posture. Donal, Gamble, and Shaw (1934) have reported a decrease but Bazett (1938) concludes that "under some conditions the reduction on standing is so slight that it falls within the experimental error." We feel justified, therefore, in assuming that in many of our experiments the cardiac output was constant or only slightly decreased and consequently the blood from the upper limbs and thorax must have been more rapidly recirculated as a result of the retarded venous return from the abdomen and lower limbs. This more rapid circulation of a decreased volume of blood through the lungs brought the blood of the upper part of the body into equilibrium with the lowered alveolar CO_2 and therefore, the CO_2 content of the venous blood taken from the hand was lowered (see table 2).

In cases where the ventilation volume was increased or remained constant in the face of the decreased alveolar CO_2 the maintenance of this lowered alveolar CO_2 was favored. However, as already pointed out, the increase in the ventilation volume cannot be looked upon as an important causative factor in the maintenance of the lowered alveolar CO_2 since with the subject FH it was equally well maintained in the face of a decreased ventilation volume.

When we recall that it is customary to look upon the alveolar CO_2 as one of the chief factors in the control of pulmonary ventilation it is difficult to understand why the ventilation volume was not decreased in all subjects. It seems likely that the impaired cerebral circulation and the decreased pressure in the carotid sinus are the factors chiefly responsible for the maintenance of a constant or increased level of pulmonary ventilation. Higgins (1914) and Main (1936) believed that their experiments indicated that some specific stimulus to respiration, probably due to afferent impulses from active muscles, resulted from standing. Since their index of increased respiratory activity was a fall in the alveolar CO_2 and since in this investigation it has been shown that changes in alveolar CO_2 may occur independent of alteration in the rate of pulmonary ventilation their evidence for such a specific stimulus to respiration must be considered unsatisfactory. Until more direct evidence is available to support the hypothesis of a stimulus to breathing by proprioceptive impulses from active muscles we feel that the assumption is unnecessary. The very moderate increases in respiratory activity which occur upon standing can very easily be explained as the result of carotid sinus reflexes and diminished cerebral circulation. The findings of Main, that sitting in a comfortable position lowers alveolar CO_2 only slightly, may be explained as due to better venous return from the limbs and less change in the functional residual air in this posture as compared with standing.

SUMMARY

Experimental evidence has been presented to show that in man the following respiratory and circulatory adjustments occur upon the assumption of the erect posture: 1, a marked drop in the alveolar CO_2; 2, the accumulation of an O_2 debt and the retention of CO_2; 3, a decrease in the CO_2 content of venous blood in the upper limbs and an increase in the CO_2 content of venous blood in the lower limbs; 4, a marked increase in the volume of the functional residual air. These changes are independent of alterations in pulmonary ventilation, which increases in some subjects and decreases in others.

The theory is advanced that the primary cause of the lowered alveolar CO_2 is simple dilution resulting from the increased volume of functional residual air, and that once established this lower level of alveolar CO_2 is maintained by an impairment of CO_2 transport from the dependent parts of the body and presumably an increased circulation rate in the arms and thorax. This results in the lower alveolar CO_2 being reflected in the venous blood of the arms. The results suggest that such changes as occur in pulmonary ventilation are the result of carotid sinus reflexes and decreased cerebral circulation and that it is unnecessary to postulate any proprioceptive stimuli to the respiratory center.

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THE ADAPTIVE VALUE OF ABSORPTION OF FATS INTO THE LYMPHATICS

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Whatever may be the *mechanism* of absorption of digested fats into the lymphatic system, there still remains the question of the *adaptive value* of this mechanism to the organism. What rôle is played in the animal economy by this peculiar indirect mode of entry of digested fats into the blood stream? What is the adaptive value of the process in which the digested fats enter the blood stream by the circuitous route of the lymphatics? The literature contains no satisfactory account of this. A common view seems to be that this phenomenon may have no adaptive significance; that, like certain other mechanisms, it possesses no positive survival value. That such is not the case, and that this phenomenon is of considerable importance, is suggested by the following experiments:

PROCEDURES. Each of ten dogs was given a mixture of olive oil and melted lard (5 grams of each, per kilo) by stomach tube. A half-hour to an hour later the dog was anesthetized with ether or with intravenous barbital. The thoracic duct was cannulated. A milky mesenteric lacteal was exposed near the intestine, and cut, allowing the absorbed material to escape and collect in little droplets on the mesenteric surface.

A single blood sample, oxalated to prevent clotting, was drawn, and used in preparing each of the following:

1. Mixtures of equal volumes (about 0.015 cc.) of blood and lacteal lymph.

2. Mixtures of equal volumes (about 0.015 cc.) of blood and thoracic duct lymph.

3. The controls: mixtures of equal volumes (about 0.015 cc.) of blood and Ringer's solution.

These mixtures were made approximately every half-hour with pipets used for ordinary white blood cell counts. Oxalated blood was drawn to the 0.5 mark. An equal volume of the liquid to be mixed with blood (i.e., lacteal lymph, thoracic duct lymph, or Ringer's solution) was drawn into the pipet behind the blood. Gentle suction carried blood plus lymph (or plus Ringer's) into the mixing chamber without spattering or bubbling, and the liquids were mixed for two minutes by shaking the pipet. Then

the mixed contents of the pipet were expelled upon a porcelain plate. Finally, an ordinary red blood cell count was made upon each mixture.

In addition, equal volumes of oxalated blood plus lacteal lymph or thoracic duct lymph or Ringer's solution were drawn into Van Allen hematocrit tubes and centrifuged at 2,200 revolutions per minute for 15 minutes. The supernatant fluid was examined for red coloration.

By these procedures we were able to determine whether lymph collected during the digestion and absorption of fats, is destructive to red blood cells.

RESULTS. Three distinct lines of evidence pointed to the existence of a hemolytic agent in the lacteal lymph and (in lesser concentration) in the thoracic duct lymph during absorption of the products of fat digestion.

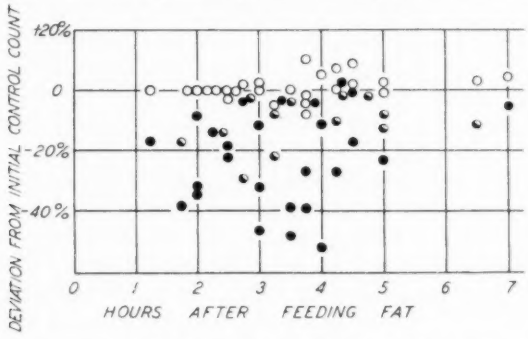

Fig. 1. Erythrocyte counts made upon blood plus Ringer's solution (open circles), blood plus lacteal lymph (black circles), and blood plus thoracic duct lymph (black-and-white circles). In each experiment the *first* control count is plotted at zero. Each subsequent count in that experiment is plotted as a percentage deviation from this figure.

1. Almost without exception the red cell counts made upon blood plus lacteal lymph were lower than the control counts. This effect was smaller both early and late in the experiments, and maximal at the time the lacteals appeared most milky, when fat absorption was most rapid. The results of these counts (upon 10 dogs) are shown in figure 1. Note that in five counts made three to four hours after fat digestion 38 per cent or more of the cells mixed with lacteal lymph were destroyed.

The results of blood counts upon blood plus thoracic duct lymph fall into two groups: Some of the counts lie between control counts and counts upon blood exposed to lacteal lymph, indicating that the substance injurious to erythrocytes tends to disappear or is diluted as the lymph courses slowly through the lymphatic vessels. In other instances the counts upon blood plus thoracic duct lymph are considerably lower than the controls, sometimes as low as the counts on blood plus lacteal lymph.

The entrance into the blood stream of thoracic duct lymph still retaining some hemolytic action is probably without significant effect, perhaps because this lymph is at once mixed with large quantities of blood returning from all parts of the body.

The averages of all counts show that lacteal lymph destroyed 21.8 ± 2.1 per cent of the erythrocytes, and thoracic duct lymph destroyed 10.8 ± 1.6 per cent. In only one dog of the series (results on which are not included in fig. 1) were the results entirely negative.

2. Centrifuged samples of blood mixed with either lacteal or thoracic duct lymph (drawn during the absorption of digested fat) showed distinct hemolysis. The supernatant fluid was tinged a definite pink, easily distinguishable from the supernatant fluid of the control tubes of blood plus Ringer's solution, in which hemolysis was very slight or absent.

3. Our observations also confirmed the finding (V. Johnson and A. Johnson, unpublished data) that mixtures of blood plus lacteal lymph or thoracic duct lymph (drawn during the absorption of digested fat) examined histologically, often show malformed erythrocytes and much debris, in which injured red blood cells can be recognized.

Discussion. From these findings we suggest that one rôle of the intestinal lymphatics and the absorption of digested fats into them is this: Erythrocytes in the slowly-moving blood of the intestinal capillaries are spared exposure to a dangerous concentration of the hemolytic material. Much of this material finds time to disappear in the lymphatic stream, or to be diluted by lymph from other sources. What remains of the hemolytic material in the upper thoracic duct is innocuous because it is suddenly diluted by large quantities of blood in the swiftly-moving subclavian stream.

What is this injurious substance? It is probably not the neutral fat itself, for mixtures of blood and milk in equal quantities give the same red cell counts as blood mixed with Ringer's solution. Furthermore, alimentary lipemia is not associated with hemolysis. The experiments of Johnson, Carlson, and Johnson (1933) suggest that the injurious substance might be glycerol. These investigators clearly differentiated between 1, the innocuous effect of feeding glycerol along with a mixed diet, in which case the glycerol may have combined with fatty acids (or perhaps amino acids), and 2, the marked hemolytic action of glycerol injected intravenously or subcutaneously. One of us has also found that the hemolysis resulting from subcutaneous injection of glycerol can be prevented or diminished by the simultaneous injection of sodium butyrate. Probably here the glycerol is rendered innocuous by combining with the soap.

These considerations, plus the experiments described, have led us to the following purely tentative hypothesis: In the course of fat digestion and absorption, some of the glycerol and fatty acid soaps are not resynthesized

to neutral fat. Absorption of this free glycerol (and perhaps soap) directly into the slowly-moving capillary blood stream might conceivably destroy large numbers of erythrocytes. By entering the lymphatics, much of this glycerol is either diluted by lymph from other than the intestinal regions or perhaps recombines with fatty acid. That glycerol which remains free when the duct lymph empties into the subclavian vein is almost immediately so diluted by blood from all the body, as to be innocuous.

SUMMARY

During the absorption of digested fat administered to dogs by stomach tube, there is a hemolytic agent in lacteal lymph collected near the intestine, and also (usually in lesser concentration) in the thoracic duct lymph. The absorption of the products of fat digestion into the lymphatics instead of directly into the blood stream probably protects against hemolysis by the toxic agent. It is suggested that this hemolytic substance might be glycerol.

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EXTRINSIC AND INTRINSIC PATHWAYS CONCERNED WITH INTESTINAL INHIBITION DURING INTESTINAL DISTENTION[1]

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Intestinal inhibition during intestinal distention has been studied in acute experiments by techniques involving anesthesia or decerebration, laparotomy, and cutting and handling the intestine (1) (2) (3). These procedures are recognized to have marked effects on intestinal motility and cannot be assumed to be without effect on the sensitivity of visceral reflexes. However, the studies are in agreement that distention of any part of the small intestine with adequate pressure results in inhibition of the small intestine both above and below the distention. Morin and Vial (3) concluded that such reflexes are dependent entirely on the integrity of the splanchnic nerves; however, intrinsic connections did not exist in their experiments since separate intestinal segments were used. Pearcy and Van Liere (1) observed no gastric inhibition during distention of the colon when extrinsic nerves were severed.

In this laboratory (4) it was found that distention of a Thiry or Thiry-Vella fistula caused inhibition of gastric motility and decreased tonus. This technique avoids the objectionable features mentioned above and we have applied it to the study of effects of intestinal distention on intestinal motility. In addition, methods have been used which permit separate study of extrinsic and intrinsic pathways for mediation of the inhibition observed.

METHODS. Sixteen dogs were used each having one or two either innervated or denervated Thiry or Thiry-Vella fistulae made from the jejunum. The method of denervation was to cut the mesentery, paint the blood vessels with concentrated phenol, and strip away the nerve fibers from the vessels. Distention of loops denervated in this way has no effect on gastric motility, causes no signs of pain, and does not produce anorexia when prolonged for days.

Intestinal activity was recorded by balloon-mercury-manometer systems, the manometer floating a writing point. The balloon and tubing were filled with water. Two separate but similar systems were used to record

[1] Aided in part by grant from the Wisconsin Alumni Research Foundation.

simultaneously the activity of separate portions of the jejunum. Routine procedure was to empty the two balloons and, having established a zero-pressure line, each was inserted into separate fistulae when extrinsic mechanisms alone were to be studied, or one balloon was inserted into the upper end and the other into the lower end of a denervated Thiry-Vella fistula when intrinsic mechanisms alone were to be studied. Use of an innervated Thiry-Vella fistula permitted study of the combined mechanisms. Once the balloons were inserted, 3 cc. of water were introduced into a side-arm of each system. After allowing a few minutes for adjustment a fairly uniform level of tonus and amplitude of rhythmic contractions is reached and is maintained in the undisturbed animal for a time considerably longer than that required for any of these experiments. Any desired pressure was obtained in either of the balloons by introduction of more water at the side-arm. Throughout these procedures the animals lay unrestrained.

The balloons used were 55 mm. long and were of sufficient diameter so that they were not stretched by the maximum amount of water introduced during a distention. Therefore, the height of the record above the zero-pressure line is a true indication of the pressure within the lumen of the intestinal segment.

Precautions were taken to prevent stretching or tugging of the parietal peritoneum. Each Thiry fistula was long enough so that the balloon did not reach to the blind end of the segment. The balloon was inserted far enough that the distention was at least an inch inward from the level where the intestinal segment was connected with the parietal peritoneum. Catheter tubing was used in the balloons to permit them to conform to the curvature of the intestinal segment. Evidence is given under results that no effects observed were referable to reflexes involving afferents in the parietal peritoneum.

Results. I. *All pathways intact.* When the distending balloon is placed in one end of an innervated Thiry-Vella fistula and the recording balloon is placed in the other end, the two are separated by tissue which is essentially normal. Neither extrinsic nor intrinsic pathways between these two points have been interrupted. Seven dogs were used for this study, and 24 experiments were done. In 15 experiments the pressure was increased posteriorly and in 9 anteriorly. The balloons were separated by from 3 to 16 cm. of undistended intestine. In each of these experiments inhibition resulted in the undistended end of the segment during distention of the other end whether anterior or posterior. Variation existed with regard to degree of inhibition. In a given animal a sudden pressure increase to a comparatively low level was more effective in producing inhibition than a gradual increase to the same or even to a somewhat higher level.

When the pressure was suddenly increased in one end of the fistula so that it was 40 to 80 mm. Hg above the zero level, sudden and complete inhibition of rhythmic contractions and lowered tonus resulted in the other end of the segment. This type of response is illustrated by figure 1. Since these effects appeared within 3 to 7 seconds they could not have been produced primarily by blood-borne substances. Maximal effects occurred during the first 30 seconds of distention although the same distention was maintained for several minutes. Tendency to break through the inhibition was evident, and in several cases a period of hyperactivity accompanied by a tonus wave occurred within the five-minute period following removal of the distention. These effects are in every way similar to those

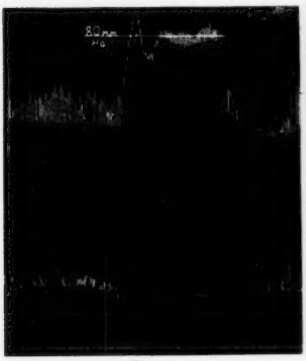

Fig. 1

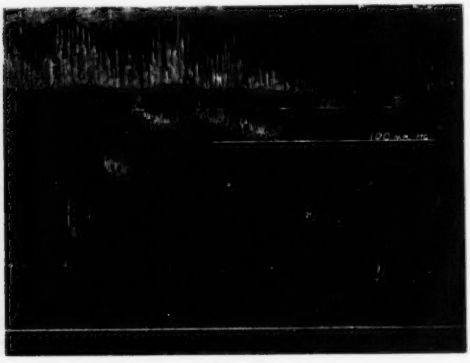

Fig. 2

Fig. 1. Effect of a sudden pressure increase in the anterior end of a Thiry-Vella fistula (upper record) on the motility of the posterior end (lower record). In this and in the following figures the straight lines indicate zero-pressure and time is shown in five second and one minute intervals or in one minute intervals only.

Fig. 2. Effect of step-by-step increase in pressure in the posterior end of a Thiry-Vella fistula (lower record) on the motility of the anterior end (upper record).

resulting from rectal stimulation (5), in which reflex inhibition of the intestine is accomplished by sympathetic nerves.

A typical result of increasing the pressure in steps is shown in figure 2. The degree of intestinal inhibition produced by this method is related to the severity of the distention, complete inhibition resulting in some animals when a sufficiently high pressure level is reached. Effective pressures varied from 60 to 130 mm. Hg.

In all experiments, except three, recovery of the intestine from inhibitory influences began immediately or within three minutes following removal of the distending pressure. In five instances the activity in the three minute period following removal of the distention exceeded that of the three minute period preceding the distention.

An effect frequently observed is illustrated in figure 3. After apparent recovery from the effects of a first distention a second of shorter duration was found to surpass the first in causing inhibitory effects. This appears to be the same phenomenon as that reported for the stomach by Lalich, Herrin and Meek (6).

II. *Extrinsic pathways cut; intrinsic connections intact.* Eight experiments were done on three dogs having denervated Thiry-Vella fistulae. In three experiments the pressure was increased in the posteriorly placed balloon, and in the remainder the pressure was increased in the anterior one. In each of the three dogs inhibition could be produced in either end of the fistula by sufficient distention of the other end (fig. 4). However, somewhat higher pressures were usually necessary and the onset of the inhibition was not sudden in any case. In two cases stimulatory effects were seen in the lower end of the segment when the pressure in the upper

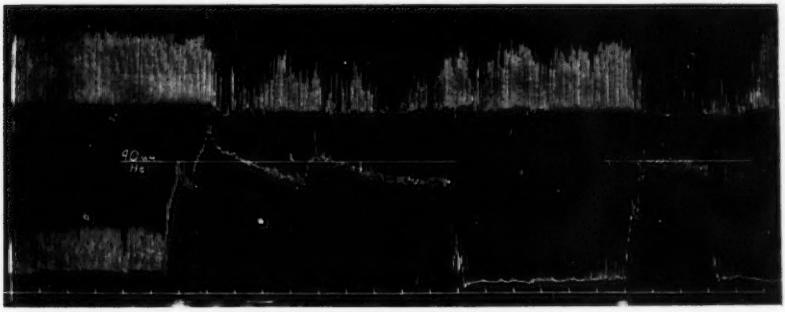

Fig. 3. Effect of successive distentions in the lower end of a Thiry-Vella fistula (lower record) on the activity of the upper end (upper record).

end was raised to 40 mm. Hg and inhibition resulted when the pressure was raised to 80 mm. of Hg. In these experiments there was a distance of 6 to 10 cm. between the two balloons. Therefore, intestinal inhibition was mediated by intrinsic mechanisms for a distance of more than 10 cm. in each direction from the locus of distention. However, the rapid phase of the intestinal inhibition resulting from distention appears to be dependent on extrinsic nervous pathways.

III. *Extrinsic connections intact; intrinsic connections cut.* By placing the two balloons in separate innervated Thiry or Thiry-Vella fistulae, the rôle of extrinsic nervous pathways in the intestinal response to distention was studied. Five dogs were used for this part of the study, and 41 experiments were done. Inhibition was clearly produced in each animal and in 33 of the experiments no matter which loop was distended, the inhibitory effects obtained were the same as those seen when all pathways were intact as described in section I. Shortness of the latent period and absence

of intrinsic connections establishes the inhibition as primarily nervous and over long reflex arcs. Afferent nerve endings activated by this procedure are located in the jejunum. The relative part played by stretch, pressure, or ischemia (7) in initiating these reflexes has not been studied. The jejunum is supplied with extrinsic nerves which inhibit its motility as a result of distention. There is no reason for doubting that these are the same sympathetic nerves utilized in the recto-jejunal reflex (5). When the pressure is gradually increased a sufficiently high level must be reached before inhibitory effects are maintained. As before, a second distention

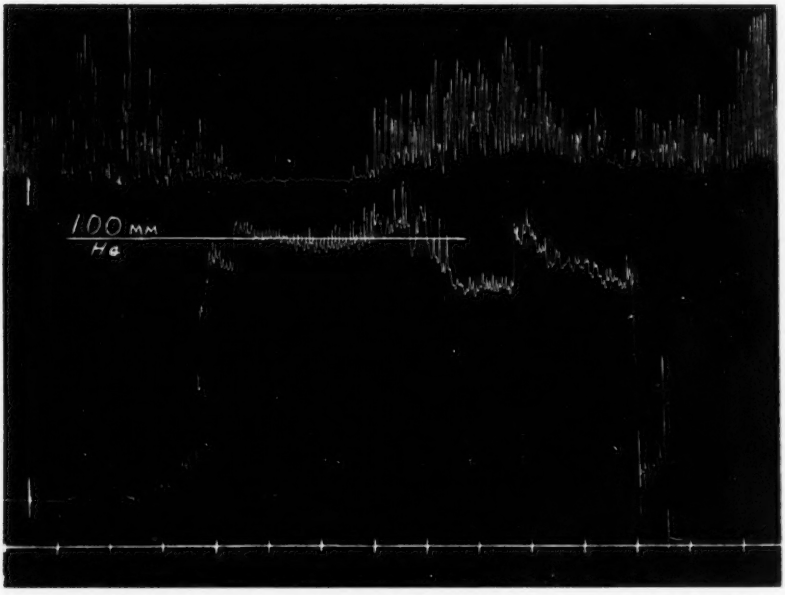

Fig. 4. Effect of distention of the posterior end of a denervated Thiry-Vella fistula (lower record) on the motility of its anterior end (upper record).

similar in magnitude to a first distention is frequently more effective. The records are similar to figures 1, 2 and 3 and have therefore not been reproduced.

In two of the dogs each having one Thiry and one Thiry-Vella fistula the effect of distending one section of the Thiry-Vella fistula on the motility of another part of the same segment (all pathways intact) was compared with effects of the same distention on the motility of the Thiry fistula (only extrinsic pathways intact). No significant differences were observed in the rate of onset or the degree of inhibition produced. Apparently the intrinsic path for mediation of intestinal inhibition is little used when extrinsic nervous pathways are intact.

These results again emphasize the nervous factor in intestinal obstruction (8). Intestinal inhibition and atony will result from intestinal distention alone uncomplicated by other inhibitory influences. Therefore, reduction of the pressure in the intestinal lumen is an essential step in permitting the intestine to recover normal motility and tonus. Wangensteen (9) has found intestinal drainage to be of value in the treatment of certain types of intestinal obstruction.

IV. *Both intrinsic and mesenteric pathways destroyed.* Four dogs each had two separate Thiry or Thiry-Vella fistulae one of which in each dog was denervated. In each of 12 experiments on these dogs, distention of

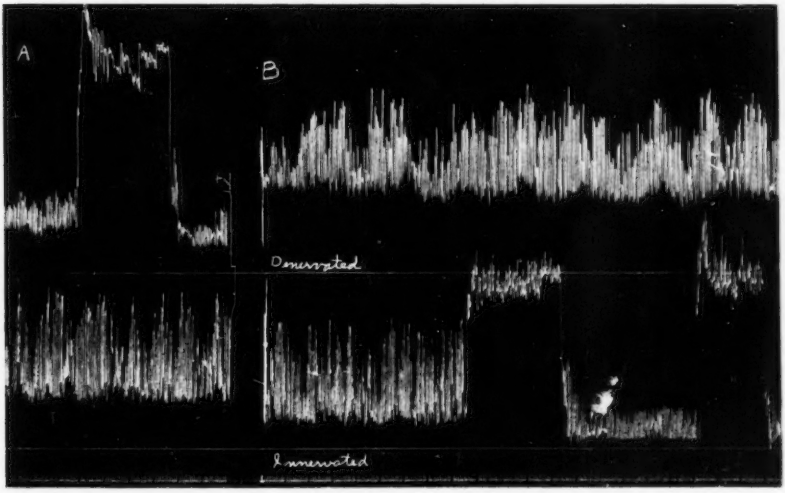

Fig. 5. A. Failure to affect an innervated Thiry fistula (lower record) by distention of a denervated Thiry fistula (upper record).

B. Failure to affect a denervated Thiry fistula by successive distentions of an innervated Thiry fistula (balloons in same fistulae).

the denervated Thiry fistula produced no effects on the motility of the innervated segment. This was true even when the pressure used was in excess of the maximum used in the previously described experiments. Figure 5 shows failure to affect the innervated segment during distention of the denervated one with a pressure of 130 to 180 mm. Hg. If any of the inhibitory effects thus far described were due to procedures employed or to stimulation of afferent endings in the parietal peritoneum rather than in the intestinal wall or the visceral peritoneum, these effects should have been seen in this type of experiment.

In eight experiments on these four dogs distention of an innervated

segment with pressures as great or greater than any previously used, except in denervated fistulae, produced no effect on a denervated segment. Figure 5b shows the ineffectiveness on the denervated segment of repeated distention of the innervated segment with a pressure of 110 to 160 mm. Hg.

Evidence has already been offered proving that the effects between intestinal segments having extrinsic pathways intact are primarily nervous, the short latent period being an indisputable part of this evidence. The experiments just described afford evidence that the inhibitory responses recorded are not even secondarily caused by blood-borne substances. In view of the present knowledge of mediation of impulses at autonomic nerve endings, the most reasonable assumption is that these extrinsic inhibitory nerves are adrenergic. They belong mainly or exclusively to the sympathetic division of the autonomic system, and the effects on the intestine are of the type reproducible by adrenalin injection (10). Since the denervated intestine is hypersensitive to the inhibitory effects of adrenalin and sympathin (10) if these substances are not produced in sufficient quantity during intestinal distention to inhibit a denervated segment then they are still further below threshold for affecting an innervated one. The question arises why the denervated intestine should not be humorally inhibited such as does occur during intestinal inhibition by rectal stimulation. In all probability fewer adrenergic nerve endings are actuated than in the widespread response to rectal stimulation.

SUMMARY

Motility of the jejunum above and below a distention has been recorded by the use of two balloon-mercury-manometer systems in experiments on unmedicated dogs having one or two Thiry or Thiry-Vella fistulae. Denervations were done to permit separate evaluation of the rôle of intrinsic and extrinsic pathways in the responses observed.

Distention of the jejunum of the dog results in inhibition of all types of movement and decreased tonus of the undistended part of the jejunum in both directions from the site of distention. The degree of inhibition depends upon the rapidity with which the pressure is increased in the balloon and upon the final pressure attained.

The inhibition of the normal jejunum as a result of distention is accomplished primarily by means of reflexes over the extrinsic nerves. The jejunum contains afferent endings which are stimulated during distention and efferent nerve endings which have an adrenine-like effect on the motor functions of the jejunum.

When the extrinsic nerves are cut an intrinsic and less efficient mechanism for mediating intestinal inhibition during intestinal distention is unmasked. This pathway is presumably over the intrinsic nerve cells of the intestinal wall.

When the extrinsic nerves are intact inhibition of the intestine during distention is accomplished as well in the absence of the intrinsic connections as when they are present.

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ELECTROCARDIOGRAPHIC CHANGES AND CONCENTRATION OF POTASSIUM IN SERUM FOLLOWING INTRAVENOUS INJECTION OF POTASSIUM CHLORIDE

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In mammals the intravenous injection of a solution of potassium chloride may produce either a sequence consisting of extrasystoles, tachycardia and finally ventricular fibrillation, or on the other hand cardiac arrest without arrythmia (5, 7, 8). Recently Nahum and Hoff (6) found in the rabbit that fibrillation was produced by the rapid injection of a concentrated solution of potassium chloride, while arrest without arrythmia occurred following slow injection of an isotonic solution. Arrest produced in this fashion is preceded by a definite sequence of electrocardiographic changes.

The present studies were undertaken to determine what relationship, if any, exists between the concentration of potassium in the serum and the various electrocardiographic changes observed following intravenous injection of an isotonic solution of potassium chloride.

PROCEDURE. Four dogs weighing from 15 to 20 kilos were employed in five experiments. Morphine sulphate, 10 mgm. per kgm. body weight, was injected subcutaneously thirty minutes before each experiment. A control electrocardiogram was obtained from lead II, and an initial sample of venous blood withdrawn under oil from the jugular or femoral vein. In three experiments (1, 2, 4 of table 1) an isotonic solution of potassium chloride (1.12 per cent) was then injected into the femoral vein at a rate approximating 10 cc. per minute, until the death of the animal. In one experiment (exp. 3 in table 1) injection was discontinued before cardiac arrest had occurred, and the animal allowed to recover. The same animal received at a later date an injection of an eight times isotonic solution of potassium chloride (exp. 5, table 1) which was continued until death occurred.

[1] Aided by a grant from the Ella Sachs Plotz fund.

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At intervals varying from two to five minutes further electrocardiograms were taken from lead II, and blood samples were withdrawn from the jugular vein at somewhat longer intervals. A final sample of blood was obtained at death from the jugular vein or from the heart. The concentration of potassium in the serum was determined by the method of Shohl and Bennett as modified by Hald (3).

TABLE 1

Summary of protocols of experiments

EXPERIMENT	WEIGHT OF DOG	CONCENTRATION OF SOLUTION INJECTED	AMOUNT OF SOLUTION INJECTED	DURATION OF INJECTION	RATE OF INJECTION	CONCENTRATION OF POTASSIUM IN SERUM	
						Time sample taken after injection	Concentration
	kgm.	*grams per cent*	*cc.*	*minutes*	*cc. per kgm. per minute*	*minutes*	*mM. per liter*
1	21.8	1.12	443	51	0.40	0	4.1
						25	8.4
						50	14.7
2	20.7	1.12	423	35	0.59	0	3.8
						30	13.2
						35	15.8
3*	15.2	1.12	383	54	0.47	0	4.9
						21	6.9
						33	8.0
						52	9.4
						65	7.9
4	15.5	1.12	472	41	0.78	0	5.5
						8	8.6
						18	9.9
						41	14.3
5	15.2	8.96	9	3	0.20	0	4.5
						2	15.8

* Atropine injected intravenously prior to experiment.

RESULTS. *Changes in concentration of potassium in serum.* In table 1 are summarized the basic data of all five experiments, including the amount of potassium chloride given, the rate of injection, the time at which each blood specimen was taken, and the concentrations of potassium in the serum at that time. It will be seen that the concentration of potassium regularly increased almost linearly with time. It was thus possible by interpolation to estimate the concentration of potassium in the serum at any given time. These interpolated values were then compared with the electrocardiographic changes as they developed.

Electrocardiographic changes. In table 2 are presented the changes most regularly appearing in the electrocardiogram, and the concentration of potassium at which they first were detected. These changes were in every way similar to those reported following slow injection of potassium chloride in the rabbit (6). Ventricular extrasystoles, ventricular tachycardia, and ventricular fibrillation were not seen, and death occurred by cardiac arrest, preceded by a characteristic sequence of changes in the electrocardiogram. From table 2 it will be clear that in all experiments each characteristic change appeared at approximately the same concentration of potassium.

First to appear was an increase in the amplitude of the T wave, usually accompanied by a shortening of its duration (fig. 2, B, C). This change became apparent at concentrations of 5.0 to 7.8 mM. per liter, and increased progressively until the T wave equalled the Q R S complex in amplitude and brevity (fig. 1, D), or until it was obscured by the onset of other

TABLE 2

Concentration of potassium in the serum (millimols per liter) at which various electrocardiographic changes appear

EXPERIMENT	INITIAL CONCENTRATION OF POTASSIUM	INCREASE IN AMPLITUDE OF T WAVE	DEPRESSION OF S-T SEGMENT	DISAPPEARANCE OF P WAVE	INTRAVENTRICULAR BLOCK	CARDIAC ARREST (DEATH)	REAPPEARANCE OF P WAVE
1	4.1	5.0	9.0	10.5	11.4	14.7	
2	3.8	7.2	7.8	10.2	10.2	15.8	
3	4.9	6.4	9.4	9.4	9.4		7.9
4	5.5	7.8	9.0	11.0	12.0	14.3	
5	4.5				10.0	15.8	

changes. Somewhat later, at concentrations of 7.8 to 9.0 mM. per liter, the S-T segment began to sink below the isoelectric line (fig. 1, C, and fig. 2, B) and continued to drop as the concentration rose (fig. 2, C). The greatest change occurred in experiment 4, in which the S-T segment fell 4 mm. below the isoelectric line when a concentration of 14 mM. per liter was attained.

The Q R S complex became definitely wider as the concentration of potassium reached 10.0 to 12.0 mM. per liter (fig. 1, E). By the time a lethal concentration of potassium was attained, this had progressed to almost complete disorganization of the complex (fig. 1, F).

The P waves first began to widen and lose amplitude at concentrations of 9.4 to 11.0 mM. per liter, and then quickly disappeared as the concentration rose further. No changes were noted in the P-R interval up to the time of extinction of the P wave. In experiment 3, in which the concentration of potassium increased most gradually, total block appeared just as the P waves disappeared (fig. 2, B). Injection of potassium was

stopped at this moment, and as the concentration of potassium dropped the P waves reappeared (at 7.9 mM. per liter) with total block between auricles and ventricles (fig. 2, D). In certain electrocardiograms (not shown here) continuous progressive decline in the amplitude of the P waves prior to their disappearance was observed. This makes it prob-

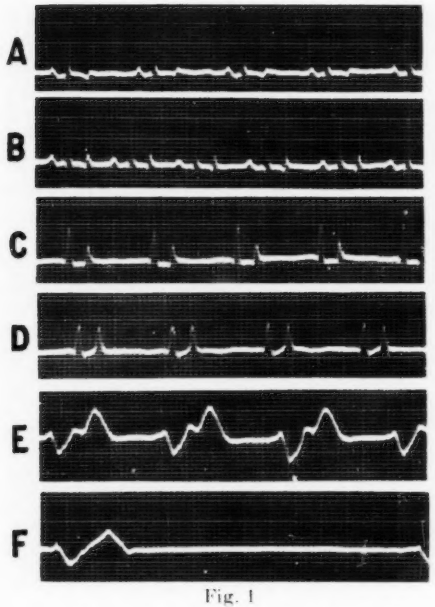

Fig. 1

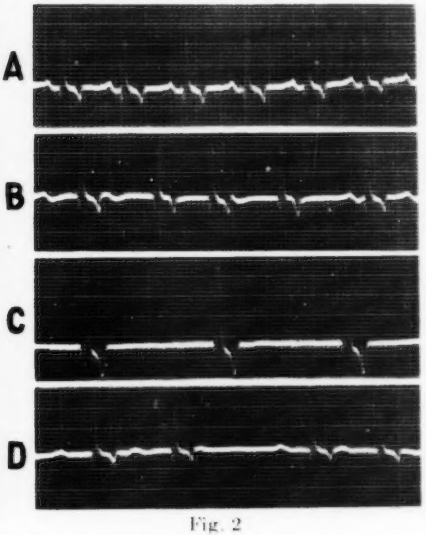

Fig. 2

Fig. 1. Serial electrocardiograms taken during intravenous injection of potassium chloride. A, control; B, increase in amplitude of T wave; C, S-T segment drops below isoelectric line, and P wave disappears; D, accentuation of previous changes; E, spreading and beginning disorganization of Q R S complex; F, complete disorganization of Q R S complex just prior to final arrest.

Fig. 2. Serial electrocardiograms showing the reversibility of electrocardiographic changes following intravenous injection of potassium chloride (expt. 3). A, normal; B, increase in amplitude of T waves; C, disappearance of P wave and drop of S T segment. At this point the injection was stopped. D shows the reappearance of the P waves, with complete heart block; also rise in S-T segment and decrease in amplitude of T wave.

able that the P waves disappear because of depression of auricular activity rather than because they are obscured by the ventricular complexes.

In two experiments a normal cardiac rate was maintained until the moment of final arrest. In one experiment a slight increase in rate was noted as the concentration reached 11 mM. per liter, in another a small decrease at the same concentration was recorded. Only in the single

experiment with an atropinized animal (expt. 3) was a progressive decline in heart rate found. Here the cardiac rate declined from 230 to 125 beats per minute as the concentration rose to 9.0 mM. per liter; at which point the normal sinus arrythmia, previously suppressed by atropine, returned.

Discussion. These experiments demonstrate a close correlation between the concentrations of potassium in the serum and the electrocardiographic changes produced by intravenous injection of an isotonic solution of potassium chloride. Failure of Harris and Levin (4) to find such a relationship in the human heart following intravenous injection of potassium chloride is consistent with our results, since in their experiments the concentration of potassium in the serum did not increase by more than 1.0 mM. per liter. Our experiments indicate that a greater increase than this is necessary in order to produce electrocardiographic changes.

It is clear from these experiments that potassium injected intravenously causes death by cardiac arrest. This is borne out by the observation that respirations and reflexes usually persisted for a brief interval after cardiac arrest and is consistent with the usually accepted interpretation of the toxic action of potassium (1). The constancy of the concentration of potassium in serum at the precise moment of cardiac arrest, 14 to 16 mM. per liter, suggests that there is a critical concentration of potassium at which the heart stops. If this is a true conception of the toxic action of potassium, then cardiac arrest should occur independently of the rate of injection or of the total amount of potassium given, except in so far as these factors combine to produce the necessary rise in the concentration in the serum. Such indeed is the case in these experiments. In certain experiments reported elsewhere (10) it proved possible to give intravenously much larger amounts of potassium than were given in any of these experiments without any harm to the animals, by injecting the material slowly enough. This conception of a fatal critical concentration of potassium agrees with the work of Hald, using isolated heart preparations (2). In experiment 3 in which the concentration of potassium in the serum rose nearly to the critical level and was then allowed to fall again, the animal recovered completely, showing that there is no delayed toxic action.

This interpretation of the toxic action of potassium explains the difference between high toxicity of the ion administered intravenously and its wholly benign character when given orally. Relatively large amounts given by mouth, such as 10 grams of potassium chloride, produce in man changes in the serum potassium concentration of only 1.0 to 2.0 mM. per liter (9). Even in subjects with advanced renal damage it is difficult to produce a rise of more than 3 mM. per liter by the oral administration of potassium salts (11). If it is justifiable to assume that the critical con-

centration of potassium in the serum at which cardiac arrest occurs in man is nearly the same as in the dog, then there is evidently a wide margin of safety after oral administration, since the serum potassium would have to increase some 9 mM. per liter to reach a fatal level.

Little can be said concerning the general biological significance of the existence of such a critical concentration of potassium. From experiments of the type reported here it is impossible to determine the nature of the critical event. It might possibly be the attainment of some particular intracellular concentration of total potassium or of some fraction of potassium, or it might involve the relationship between the intracellular and the extracellular concentrations. In the absence of experimental data bearing directly on this problem such speculations must be deferred. The electrocardiographic evidence is of little help, since it indicates only that the crucial event causing arrest is a complete disorganization of the ventricular complex and a disappearance of the normal action current. Such disorganization is not characteristic of potassium poisoning alone, but has been reported in cardiac arrest from a variety of causes.

SUMMARY

1. Potassium chloride in isotonic solution was injected intravenously into dogs at relatively slow rates. Serial electrocardiograms were taken from lead II, and blood samples withdrawn at intervals throughout the experiment to permit determination of the concentration of potassium in the serum.

2. Alterations in the T wave regularly appeared at concentrations of 5 to 7 mM. per liter, depression of the S-T segment appeared at 8 to 10 mM. per liter of potassium in the serum. Intraventricular block began at concentrations of 10 mM. per liter, the P waves disappeared at 9 to 11 and cardiac arrest took place at 14 to 16 mM. per liter.

3. No ectopic arrythmias of any type were observed at these rates of injection.

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THE MAINTENANCE OF EMBRYO LIFE IN OVARIECTOMIZED RABBITS[1]

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In a recent review Allen (1937) presented data showing that the injection into rabbits ovariectomized 20 hours after fertile mating of oestrin-free progestin or crystalline progesterone in dosages up to 1.5 mgm. a day maintained implantations until the 11th day of pregnancy in 3 out of 12 cases, and in only one case carried the fetuses until the 20th day. Crude corpus luteum extracts in similar (or even lesser) unitage (Corner and Allen, 1929) carried embryos through to the time of parturition. The obvious deduction appeared to be that the crude extracts contained substances other than progesterone which are necessary for the maintenance of the last two-thirds of pregnancy. Oestrin injected in combination with crystalline progesterone, in either the amounts present in the original extracts or in even larger amounts, did not completely suffice as the needed adjuvant although certain combinations were more successful than progesterone alone. The suggestion that progesterone is the hormone of pseudopregnancy, but not alone sufficient for inducing mid- and late gestational uterine conditions has been made by others (Ostergaard, 1937) and Korenchevsky and Hall (1937) claim that in the rat a proper balance of progesterone, testosterone, and oestrone is necessary for full gestational development. We have advanced the thesis that progesterone is rather rapidly destroyed (or converted into an inactive form) particularly in the ovariectomized rabbit (Pincus and Zahl, 1937) and that this conversion is inhibited by various oestrogenic substances (Pincus, 1937; Werthessen and Pincus, 1938). Fels (1935) had already demonstrated an enhancement of progesterone action by the inactive pregnandione. Conceivably, then, the efficacy of the crude extracts of Allen and Corner might depend not upon specific uterus stimulating substances, but merely upon the fact that these extracts contained substances that prevented progesterone destruction, so that sufficient amounts remained in the circulation to permit the normal progestin effects. It is of course known that crude corpus luteum extracts contain, besides oestrin, inactive compounds

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closely related to progesterone (Slotta, Ruschig and Fels, 1934; Wintersteiner and Allen, 1934; Fieser, 1936, et al.). In addition the original Corner-Allen extracts might have contained androgens.

Since we have already shown that oestrogens will enhance progesterone action presumably by inhibiting its destruction we will, in this paper, present data demonstrating similar enhancement by androgens and by the inactive pregnandiol. Furthermore, we will show that the whole course of pregnancy can be maintained by crystalline progesterone alone.

METHODS. For the demonstration of the enhancing effect of androgens and pregnandiol we have used the standard technique previously described (Pincus and Werthessen, 1937; Pincus, 1937; Werthessen and Pincus, 1938). Briefly recapitulated, rabbit does ovariectomized at 18 to 20 hours after a fertile mating are injected subcutaneously twice a day with a standard dosage of progesterone and intraperitoneally with the second compound for 3 days beginning the morning after ovariectomy. On the 5th day the female is sacrificed, the uterus flushed, and the ovum diameter measured; a cross-section of the uterus, fixed in Bouin's solution and stained with Ehrlich's hematoxylin, is measured by a planimeter for total mucosal (M) and glandular (G) areas and the ratio G/M is taken as the proliferation index. We can tell whether the egg diameter, the proliferation index, or the two combined in a measurement (Z) differ significantly from the expected values for the dosage of progesterone employed by comparison with our standard data (Pincus and Werthessen, 1937).

In the longer injection periods animals ovariectomized as described were injected twice daily with given progesterone dosages and either sacrificed or the uterus examined after laparotomy on the 11th day of gestation. Any implantations present were measured (see table 2) in longest and shortest diameters. Whenever possible a section of the uterus was taken for measurement of the proliferation index. Injections were continued twice daily after laparotomy in a number of cases and the presence of embryos determined by palpation from time to time. In rabbits carried to term injections were discontinued on the 28th day. Administrations by unction were done by implanting crystals or tablets as indicated in table 2.

Only crystalline material was employed and the injections were made with olive oil solutions.

RESULTS. The data on the short time injections are presented in table 1. They indicate that: 1, testosterone proprionate injected intraperitoneally in the dosages employed does not significantly enhance the effects of progesterone, but it does do so when injected subcutaneously (rabbit 4); 2, testosterone proprionate alone in the highest dosage employed does not act as a progestin (rabbit 5); 3, methyl testosterone injected subcutaneously significantly enhances the progesterone effect (rabbit 7) and

injected alone acts as a progestin, giving in 21 mgm. dose a Z index equivalent 0.7 mgm. progesterone, i.e., it is 1/30th as effective as progesterone; 4, pregnandiol enhances the progesterone effect significantly in one animal (10) and very nearly so in another (9), the mean data for the two animals indicating an enhancement of over 50 per cent. The inefficacy of the intraperitoneal testosterone proprionate injections may be explained on the assumption that the intraperitoneal route makes for such rapid absorption and excretion (or inactivation) that the effects cannot be expressed. This is exemplified by the results with rabbit 11 which should have given an ovum diameter of 610 micra and a G/M value of 0.58 for the dosage

TABLE 1

The effects of progestin, testosterones and pregnandiol in various combinations upon the early course of pregnancy in ovariectomized rabbits

RABBIT NUMBER	PROGESTERONE TOTAL DOSAGE	SECOND COMPOUND	TOTAL DOSAGE OF SECOND COMPOUND	MEAN EGG DIAMETER MICRA‡	G/M‡	Z‡	NUMBER OF CORPORA LUTEA	NUMBER OF EGGS
	mgm.		*mgm.*					
1	0.42	Testosterone proprionate	6.0*	528	0.393	66.93	8	1
2	0.42	Testosterone proprionate	9.0*	580	0.517	83.33	7	4
3	0.42	Testosterone proprionate	12.0*		0.512		5	0
4	0.42	Testosterone proprionate	12.0†		*0.848*		8	0
5	0.00	Testosterone proprionate	12.0*		0.222		7	0
6	0.42	Methyl testosterone	10.0†	620	0.440	76.12	5	5
7	0.42	Methyl testosterone	15.0†		*0.688*		8	0
8	0.00	Methyl testosterone	21.0†	445	0.734	102.74	12	8
9	0.42	Pregnandiol	15.0*	620	0.574	91.53	14	5
10	0.42	Pregnandiol	15.0*	*1012*	*0.633*	*114.45*	8	5
11	0.60*			**245**	**0.184**	**30.24**	10	4

* Intraperitoneal injection.

† Subcutaneous injection.

‡Italic figures indicate significant enhancement of progesterone effect, boldface figures indicate significant inhibition.

employed if injection were made subcutaneously, but gave, after intraperitoneal injection, values which did not differ significantly from the uninjected ovariectomized level of our standard curve.

In table 2 we present our findings for animals receiving various administrations for eleven days or longer. It should be noted that increasing the progesterone dosage results in an increase of the proliferation index on the 11th day, but that implantation does not occur until a dosage of 1 mgm. a day is employed. Both oestrone (rabbit 16) and pregnandiol (rabbit 17) increase the effects of a 0.5 mgm. per day injection, but apparently not sufficiently to insure implantation. The G/M ratio for normal 11-day

TABLE 2

Progestin injections and the later course of pregnancy in ovariectomized rabbits

RABBIT NUMBER	PROGESTERONE DOSAGE	SECOND COMPOUND	DAY OF PREGNANCY KILLED OR BIOPSIED	MEAN IMPLANT DIAMETER	G.M.	NUMBER OF CORPORA LUTEA	NUMBER OF IMPLANTS
	mgm./diem	*mgm./diem*		*mm.*			
12	0.17		12		0.264	13	0
13	0.17		12		0.275	7	0
14	0.50		11		0.492	5	0
15	0.50		11	Deciduomata	0.596	7	0
16	0.50	Oestrone* 0.003	11		0.600	5	0
17	0.50	Pregnandiol† 0.50	11		0.639	10	0
18	1.00		11	All living = 10.3 x 15.1		7	7
19‡	1.00		13	All living = 14.4 x 12.2		5	5
20	2.00		11	6 living, 1 dead	0.712	9	7
21	2.00		11	3 living = 15.7 x 12.7, 3 dead	0.681	6	6
22	1.00		11	2 living = 15.5 x 13.5, 3 dead = 8.3 x 6.0		7	5
22	1.00		20	2 dead left out of 5 at 11th day	0.562	7	2
23	1.00		11	4 living = 15.5 x 15.5, 2 dead		8	6
23	1.00 for 10 days, 2.00 for next 17 days		33	1 dead in vagina, 2 degenerate in uterus		8	3
24	1.00		11	2 living = 17.0 x 14.5, 1 dead = 5.0 x 5.0		13	3
24	1.00 for 10 days, 2.00 for next 16 days		27	3 dead in uterus		13	3
25	1.00		11	5 living = 13.4 x 12.0		7	5
25	1.00 for 10 days, 2.00 for next 17 days		35	2 born alive 100 and 85 gm., 1 born dead 75 gm.		7	3
26	15 mgm. intramuscular		11		0.498	7	0
26	15 mgm. intramuscular		20		0.424	7	0
27	0.00	17-aethinyl testosterone 15 mgm. in peritoneum	11		0.151	7	0
28	0.00	17-aethinyl testosterone 15 mgm. in peritoneum	11		0.167	6	0

* Subcutaneous injection.

† Intraperitoneal injection.

‡ Sacrificed because ill.

pregnant rabbits is 0.73 (Pincus, 1937). It is interesting to note that on the basis of our standard curve this value should certainly be attained on the 5th day by the injection of 0.5 mgm. progesterone per day, so presumably full stimulation is not maintained to the 11th day. Similarly the 0.17 mgm./diem dosage gives a mean value of 0.27 on the 12th day whereas on the 5th day this presumably was 0.46. This decline in proliferative activity has also been described by Allen (1937) and Ostergaard (1937). The data on rabbit 22 indicate that 1 mgm. of progesterone per day will not maintain living embryos till the 20th day (note that the G/M value has fallen to 0.562). When on the 12th day the progesterone dosage is raised to 2 mgm. per day one or more fetuses are carried to term in two out of three rabbits (23 and 25). The intramuscular implantation of 15 mgm. of progesterone crystals (rabbit 26) did not maintain pregnancy but sustained the endometrial proliferation above the ovariectomized level of 0.18; 17-aethinyl testosterone in 15 mgm. dose (as Proluton C tablets) was completely ineffective when implanted in the abdominal cavity (rabbits 27 and 28).

Discussion. While these data demonstrate that progesterone alone in sufficient dosage will carry rabbit embryos through to term, they do not definitively resolve the problem of the difference between the effects of crude corpus luteum extracts and crystalline progesterone. They do to some extent substantiate our suggestion that the difference merely lies in the inhibition of progesterone destruction by the inactive substances present in the crude extracts. The six animals receiving 1 mgm. a day till the 11th day had had a total dosage of 9.5 mgm. before laparotomy. This is exactly the dosage that 5 of Allen's (1937) rabbits received, yet only 1 of these 5 had implantations. The two animals injected with 2 mgm. per day till the 11th day received a total dosage of 19 mgm., only 4 mgm. more than the 7 rabbits in Allen's series of which 3 had implanted embryos. Although not explicitly stated in his paper we judge he followed the standard Allen-Corner procedure of one daily injection, whereas we inject twice daily. It is entirely reasonable to suppose that this dividing of the dose allows a more effective steady supply of active hormone, i.e., less hormone destroyed. This is substantiated by the fact that our standard assay method gives the equivalent of Allen-Corner +++ to ++++ endometrium values with lower dosages. Furthermore, it is notable that Allen's higher dosages were most effective. If implantation requires special substances then crystalline progesterone should be ineffective at any dose and should not increase the per cent implanted with increasing dose, but give a constant per cent at all doses unless specific breakdown products of progesterone are the needed adjuvants. The only alternative is to suppose that in our operations we tend to leave behind small ovarian fragments that contribute adjuvant secretions. We cannot accept this alternative

because no such fragments have ever been detected, and are practically automatically precluded by our methods of excision (see addendum).

We did not employ androgens in the long time experiments because their definite progestin effect in the rabbit (Klein and Parkes, 1937; Robson, 1937) is established only for the progestational period. In one experiment (rabbit 17) pregnandiol in rather low dose did act as an enhancer of the progesterone effect by the 11th day, but our limited supply of pregnandiol prevented a more thorough exploitation of this phenomenon. If our general theory is correct then it may be suggested that Allen should have been able to maintain pregnancy with oestrone-progesterone combinations, but as he himself points out oestrone has a specific involuting effect on the endometrium so that a properly balanced dosage combination can be attained only with much careful trial. We have already shown that the progesterone-sparing action of the native oestrogens is observable only with low dosages in the 5-day test, the typical anti-progesterone action occurring with higher oestrin doses.

Our data do indicate that progesterone alone is the hormone of pregnancy provided it is administered in sufficient amount. It was once thought that the typical progestational effect in a long-time castrated rabbit could only be had after preliminary priming with oestrin (*cf.* Hisaw, 1932), but more recent evidence (Hisaw, Greep and Fevold, 1937) shows that if the progesterone dosage is markedly increased the typical progestin effect may be had.

SUMMARY AND CONCLUSIONS

1. The data on ovariectomized rabbits examined at the 5th day after mating are taken to show that testosterone proprionate and methyl testosterone enhance the effect of progesterone when the former are given in certain dosages by the subcutaneous route. The enhancement may be due either to a prevention of progesterone destruction or to a demonstrable progestin-like action of the androgens themselves. Pregnandiol is similarly enhancing.

2. The data on progesterone injection for eleven days and longer indicate that normal implantation will occur with a dosage of at least 0.5 mgm. given twice daily (8 animals) and that a dosage of 1.0 mgm. twice daily after the 11th day will maintain the embryos to term (2 out of 3 animals).

3. Crystalline progesterone in 15 mgm. total dose implanted intramuscularly will maintain a moderate degree of pseudopregnant proliferation whereas 15 mgm. of 17-aethinyl testosterone will not do so when implanted intraperitoneally.

4. It is inferred that one need not invoke any special gestational substances other than progesterone for maintaining full gestation in the ovariectomized rabbit, but substances related to progesterone and not them-

selves progestins (e.g. pregnandiol, oestrone) may effectively enhance the typical progesterone effect by preventing the rapid destruction of progesterone.

This investigation was carried on in the Animal Research Station while we were guests of the Director, Dr. John Hammond. We are grateful to him for his generous hospitality. To Dr. A. S. Parkes we express our indebtedness for the methyl testosterone used in this investigation. We are much indebted to Dr. A. Neumann, of Schering, Ltd. for the supplies of testosterone proprionate, pregnandiol, and progesterone.

ADDENDUM: Animal number 25 suckled her young for 30 days. Upon autopsy shortly thereafter a completely atrophic uterus was found, indicating total absence of endogenous ovarian hormone.

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AN ANALYSIS OF THE FACTORS INVOLVED IN THE VARYING EFFECTS OF CARBON DIOXIDE UPON RESPIRATORY RATE[1]

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An increase in the carbon dioxide of the inspired air most commonly produces a distinct increase in the depth of breathing with little change in rate. A distinct slowing or a marked acceleration may, however, occur. In a single series of experiments upon dogs with intact vagi and with the same type (morphine urethane) and approximately the same depth of anesthesia, we have found a 12 per cent to 14 per cent carbon dioxide mixture to cause changes in respiratory rate varying from an 85 per cent slowing to a 204 per cent acceleration. If the respiratory effect of carbon dioxide were dependent upon the action of the gas upon one mechanism alone, one would certainly expect a greater degree of constancy of effect than is observed. However, when one considers the fact that carbon dioxide stimulates the respiratory center, augments the discharge of peripheral chemoceptors and depresses reflex activity in general including vagal respiratory reflexes (Gesell and Moyer, 1935) it is not surprising that the resultant of these various effects is not constant. The picture is further complicated by the fact that the resultant respiratory effect of any procedure need not represent simply the algebraic sum of the effects upon the various individual structures involved. Thus, Gesell, Steffensen and Brookhart (1937) found that combined chemoceptor stimulation and vagal stimulation produced marked acceleration of respiration, while the former alone produced only a mild increase in respiratory rate, and the latter an actual slowing.

Since an important part of the central respiratory mechanism is apparently located very superficially in the floor of the fourth ventricle in the region of the calamus scriptorius (Nicholson, 1936) we felt that some information regarding the mechanism of CO_2 action might be obtained by studying the effects of irrigation of this region with Locke's solution equili-

[1] These experiments were supported in part by a grant from the Rockefeller Foundation to Robert Gesell for studies on respiration.

[2] A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, in the University of Michigan.

brated with CO_2 at various tensions. In our first experiments the Locke's solution at body temperature was equilibrated with 100 per cent CO_2 at atmospheric pressure or a mixture of 79 per cent CO_2 and 21 per cent O_2. In contrast to the usual lack of marked change in rate from intrapulmonary administration, CO_2 administered in this way ordinarily caused a decided slowing of respiration. Figure 1 A is a typical example. Occasionally a slight acceleration was observed, but this was the exception and not the rule. In subsequent experiments the effect of varying the tension of carbon dioxide in the irrigating fluid was studied. In figure 1 C, 15 per cent CO_2 decreased the respiratory rate from 19 to 15 per minute (21 per cent) while in 1 D 79 per cent carbon dioxide in the same animal decreased the rate from 20 to 13.5 per minute (32 per cent). Figures 1 E and 1 F are from one of the rare experiments in which medullary

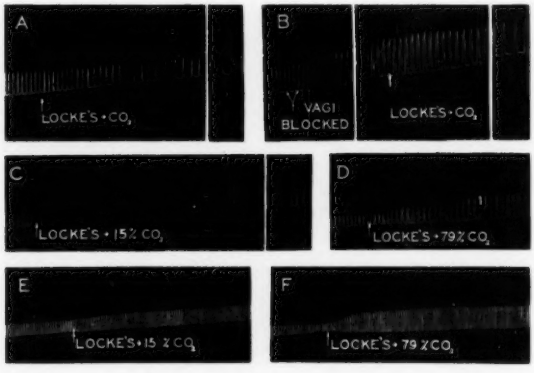

Fig. 1. Respiratory records in this and subsequent illustrations are spirometer tracings, upstroke representing inspiration.

irrigation with carbon dioxide increased the respiratory rate. In 1 E, 15 per cent carbon dioxide increased the respiratory rate from 28 to 34 per minute (21 per cent) while in 1 F, 79 per cent carbon dioxide increased the rate from 21 to 27 (29 per cent). It is evident then that this predominant tendency of carbon dioxide when applied to the floor of the fourth ventricle to slow respiration is relatively independent of the tension of the gas, a variation in tension varying the magnitude, but not the direction of the change.

Since the nucleus of the fasciculus solitarius lies near the surface of the brain stem in the region irrigated and since it has been shown that vagal reflexes may be abolished by cooling this region (Nicholson and Brezin, 1937) or applying cocaine to it (Nicholson and Sobin, 1938), this slowing of respiration by carbon dioxide might be purely the result of depression

of vagal reflexes. This possibility is rendered unlikely by the character of the slowing, which is to a considerable extent due to an increase in the duration of the expiratory pause while the slowing of respiration from vagotomy is due largely to an increase in amplitude and a decrease in the speed of inspiration. Furthermore, irrigation of the floor of the fourth ventricle with carbonated Locke's solution causes equal respiratory slowing after vagotomy. Figure 1 A shows a fairly typical response to irrigation with 100 per cent carbon dioxide. The respiratory rate decreased from 18 to 10 per minute, or 44 per cent. Vagotomy (fig. 1 B) slowed the rate from 19 to 14 per minute (26 per cent). Reëxposure of the calamus region to the same carbon dioxide solution now slowed the rate from 14 to 8 per minute, again a reduction of 44 per cent. This of course does not

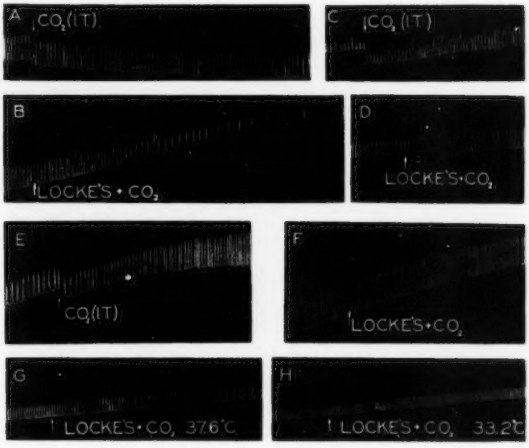

Fig. 2

mean that in the intact animal depression of vagal reflexes may not be an important factor in hypercapnic slowing. It may mean simply that following vagotomy other reflexes, also of course susceptible to hypercapnic depression, are now dominant.

Our next procedure was to compare the effects of intratracheal administration of CO_2 (12 to 14 per cent) with the effects of irrigation of carbonated Locke's solution in the same animal. When intratracheal administration of CO_2 decreased the respiratory rate, irrigation decreased the rate to a much greater extent. This difference is well shown in figures 2 A and B. In those cases in which intratracheal carbon dioxide caused a slight or moderate acceleration the usual effect of irrigation was a slight slowing or no change in rate. In figure 2 C intratracheal carbon dioxide

increased the respiratory rate from 15.5 to 17 per minute, a 16 per cent increase, while irrigation with Locke's solution plus carbon dioxide (2 D) failed to alter the respiratory rate. In those cases in which intratracheal carbon dioxide caused a very marked acceleration of respiration, irrigation might cause a slight acceleration. In figure 2 E, intratracheal carbon dioxide increased the respiratory rate from 11.2 to 23 per minute, 104 per cent, while in 2 F from the same animal irrigation increased the rate from 19.6 to 25 per minute, a 27 per cent increase. In a few experiments the effect of administration of carbon dioxide simultaneously by the two methods was determined. In some cases irrigation was initiated during the course of an intratracheal administration. In other cases the two procedures were carried out in the reverse order. The combined effect of the two was invariably a slowing of respiration, usually considerably more marked than one would expect from addition of the two individual effects.

From the above results it seems evident either that carbon dioxide centrally applied compared with intratracheal administration exerts a disproportionate effect on the same factors of the central integrative mechanism or that new types of action are open to irrigation not available to ordinary intratracheal administration. The former possibility seems the more likely though a combination of the two should be considered. In previous work we have demonstrated the presence of a mechanism in the region of the calamus scriptorius which is apparently responsible for interrupting inspiration and bringing about expiration. This mechanism seems to be readily depressed by cooling (Nicholson, 1936) and by cocaine (Nicholson and Sobin, 1938) and is apparently stimulated by the local application of nicotine (Nicholson and Sobin, 1938). In view of these results it seems not unlikely that the *marked* decrease in respiratory rate resulting from irrigation of the floor of the fourth ventricle with solutions containing carbon dioxide may be due in part at least to a stimulation of this expiratory mechanism by the carbon dioxide. If this is the case, one would expect that depression of this expiratory mechanism by cooling should diminish or abolish the slowing effect of carbon dioxide and we have found this to occur. In figure 2 G, irrigation of the floor of the fourth ventricle with carbonated Locke's solution decreased the respiratory rate from 30.5 to 23 per minute (24 per cent). In this experiment the Locke's solution, as was ordinarily the case, was at body temperature. The temperature of the Locke's solution was then lowered to 33.2°C and although equilibrated with the same tension of carbon dioxide (79 per cent) now had no effect on respiratory rate (fig. 2H). (It is of interest in this connection that Nicholson and Brezin (1937) observed a marked exaggeration of the accelerating effect of intratracheal carbon dioxide as a result of moderate cooling of the calamus scriptorius.)

Time relations of the respiratory cycle. More direct information as

to the mode of action of CO_2 is afforded by examination of various phases of the respiratory cycle. While in the preceding sections, attention was drawn to the differences between the effects of CO_2 administered intratracheally and by irrigation, the phase relationships in the two cases are essentially of the same nature, and thus to a great extent independent of the mode of carbon dioxide administration. Respiration has been long divided into two phases, inspiration and expiration, the latter phase subdivided into a stage of movement of air, and one when the chest is in the position of expiratory arrest. Gesell (1936), in studying potentials of inspiratory and expiratory muscles, drew attention to the fact that expiration is in part determined by persistence of inspiratory discharges, and from further evidence to be summarized later, we have found it convenient to consider as one period inspiration plus the first phase of expiration. This we have referred to as the period of *inspiratory stress*, as opposed to the expiratory pause. Graphically it represents the period

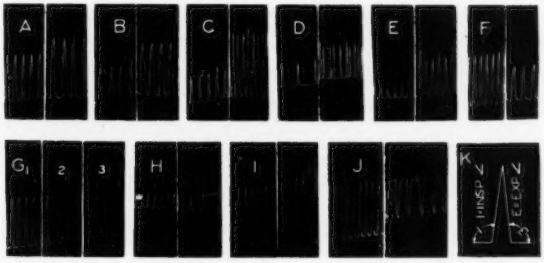

Fig. 3

between the departure of the curve from the base line and its return. Changes in inspiratory stress can be brought about by either changes in the duration of the inspiratory movement, in the length of time inspiration is maintained, or in the time that air is leaving the lungs, or any combination of these.

In all cases where the response to increased alveolar carbon dioxide or irrigation of carbon dioxide over the floor of the fourth ventricle manifested itself by a decrease in the respiratory rate, the period of inspiratory stress increased. The expiratory pause, however, was variable in this type of response. In less than half of the total observations did it increase. In figure 3 A, a respiratory slowing of 15 per cent occurred; inspiratory stress increased by 74 per cent and the expiratory pause decreased 38 per cent.

When hypercapnia produced an increased respiratory rate, the expiratory pause invariably shortened. Changes in inspiratory stress were in part determined by the degree of hyperpnea produced. When the acceleration was marked, the decrease in expiratory pause was aided by a

decrease in inspiratory stress, both contributing to a more rapid respiratory cycle. On the basis of the total observations of an increased respiratory rate, 50 per cent showed an increase in inspiratory stress, 20 per cent exhibited little change, and 30 per cent decreased. These various types of response are illustrated in figures 3 B, C and D. In figure 3 B there was an increase in respiratory rate of 21 per cent due entirely to a 66 per cent decrease in the duration of the expiratory pause, the period of inspiratory stress actually increasing 55 per cent. In 3 C the respiratory rate increased 100 per cent with no change in the duration of the period of inspiratory stress, while in 3 D there was an acceleration of 181 per cent to which both the expiratory pause and the period of inspiratory stress contributed, the former by a decrease of 61 per cent and the latter by a decrease of 39 per cent.

It is of interest that in many cases where CO_2 produces little or no change in the total respiratory rate, marked changes in phase relationships may occur. In figure 3 E, CO_2 produced no change in rate, but while in the normal cycle inspiratory stress occupied 30 per cent of the cycle, during the hypercapnic state it occupied 72 per cent of the cycle. Thus a 138 per cent increase in inspiratory stress counterbalanced a 68 per cent decrease in expiratory pause.

These observations leave no doubt as to the inadequacy of the statement of Hammouda and Wilson (1932) that the sum of the duration of the first two phases of respiration is relatively constant under various experimental conditions, variations in the respiratory rate resulting almost entirely from variations in the duration of the expiratory pause.

Form of the respiratory cycle. In 1935 Gesell and Moyer introduced the term "chemical vagotomy" for the relatively slow breathing produced by depression of the vagal reflex under exposure to CO_2, this being a specific application of the depressant effects of CO_2 on spinal cord reflexes (Glazer, 1929). Carbon dioxide administration is not infrequently accompanied by a marked slowing of the speed with which inspiration proceeds. This may readily be detected by measuring the angle which the inspiratory tracing makes with the base line, angle I in figure 3 K. In figure 3 F carbon dioxide irrigation decreased the respiratory rate from 21 to 13.5 per minute and increased this inspiratory angle from 93° to 94.7°. Examination of this record further shows that this slowing of inspiration becomes more marked as inspiration approaches its peak. Nicholson and Brezin (1937) pointed out that the most characteristic effect of vagotomy on breathing is the increase in duration of the inspiratory phase, and Gesell, Steffensen and Brookhart (1937) demonstrated an *acceleration of inspiration* with electrical stimulation of the vagi. Thus the decrease in the speed of inspiration by CO_2 can be interpreted as vagal depression.

However, this slowing of inspiration in hypercapnia is not the most

common finding. More frequently carbon dioxide has just the opposite effect; namely, a quickening of inspiration. This effect is well illustrated in figure 3 G where the normal respiratory cycle (G_1), the response to a medullary irrigation of carbon dioxide (G_2), and the additive effects of an intratracheal carbon dioxide administration (G_3) have in the order given, inspiratory angles of 92.1°, 91.6° and 90°. This increased speed of inspiration might conceivably be due to either of two causes: 1, a central blocking of the restraining effect which afferent impulses may have on inspiration, or 2, a stimulation of an inspiratory mechanism. Barcroft (1934) believes that the gasp, which consists of an abrupt swift inspiration terminating suddenly in an abrupt swift expiration, represents the fundamental unit of respiration and that it appears upon complete removal of all the factors inhibiting respiration. If this is the case, blocking of inhibiting impulses might be involved in this acceleration of inspiration by carbon dioxide. Barcroft emphasizes the depressant action of carbon dioxide upon nervous processes stating that, "It is much easier to conceive of carbon dioxide as paralyzing a nervous process than as stimulating it," and again, "that carbon dioxide acted like making suitable cuts through the medulla." However, we are inclined strongly to the view that the increased speed of inspiration frequently seen in hypercapnia is the result of inspiratory stimulation. In the first place there is little or no evidence in favor of the view and considerable fairly direct evidence against the view that removal of afferent impulses favors gasping respiration or an increase in the speed of inspiration. In the second place, in the experiments of Samaan and Stella (1935) upon the discharge of carotid gland receptors we have direct evidence of stimulation of a nervous process by carbon dioxide. Finally, we are forced to the view that carbon dioxide is stimulating inspiratory activity by a consideration of the effects of CO_2 on the speed of expiration.

Expiration in the hypercapnic state, when compared with the normal is markedly slowed. Thus in figure 3 G, the normal expiratory angle is 92.5°; irrigation with CO_2 increased the angle to 95° while addition of intratracheal carbon dioxide further increased it to 97°. It will be recalled that the total period of inspiratory stress invariably increased when CO_2 caused a slowing of respiration and increased in a considerable number of the cases when CO_2 caused an increase in respiratory rate. *The most important factor involved in increasing the duration of the period of inspiratory stress apparently is this decrease in the speed of expiration.* This marked slowing of expiration can only be explained by a persistence of activity in the inspiratory muscles, further evidence we believe of inspiratory stimulation by CO_2. That activity in inspiratory muscles may disappear only gradually as expiration proceeds has been amply demonstrated by Gesell (1936) as he says in the discussion of electromyograms, "final deflation was only accomplished as inspiratory potentials faded from

the record." In addition, recent work of Gesell and White (1938) has shown that in cyanide hyperpnea there is a long after-discharge of inspiratory muscles, and with it, a marked slowing of mechanical expiratory effort.

Apneustic respiration. This type of respiration, breathing characterized by a tendency to hold the breath in the inspiratory position, may be seen in hypercapnia. Although apneustic breathing was observed as early as 1880 (Marckwald and Kronecker) Lumsden (1923) reinvestigated the problem by means of sectioning the brain stem, and arrived at his well known series of phylogenetic centers stretching from the calamus to the upper pons. Subsequently, apneustic breathing has been produced in a variety of ways—by section of the rubrospinal tracts plus section of the vagi (Henderson and Sweet, 1929), by local cooling of the floor of the fourth ventricle (Nicholson, 1936), by hydrocyanic acid gas (Taylor, 1930), by carbon dioxide (Barcroft and Margaria, 1932) and by cocainization of the floor of the fourth ventricle (Nicholson and Sobin, 1938).

Figures 3 G, H and I illustrate various types and degrees of apneustic breathing seen with carbon dioxide administration. Figure 3 G_3 as contrasted with figure 3 G_1 shows a slight but definite pause at the height of inspiration (as well as a more abrupt inspiratory movement and greatly delayed expiratory movement). In figure 3 H the carbon dioxide was administered by irrigation of the floor of the fourth ventricle while in 3 I it was administered intratracheally. A definitely prolonged inspiratory pause is in evidence in both of these records, as well as the other changes mentioned above.

From purely theoretical considerations, it is apparent that maintained inspiration can result from either 1, the inability of some normally occurring inhibitory process to interrupt inspiration, or 2, intense and prolonged stimulation of an inspiratory mechanism. Of all the methods used to produce apneusis, central cooling is perhaps the one that most certainly produces depression with no concomitant stimulation. In figure 3 J we see an example of apneustic respiration resulting from local cooling of the surface of the brain stem. When we examine this record we see that inspiration is actually slower than in the normal record, the tracing sloping off more and more as the ascent continues, presenting a distinctly rounded appearance as it approaches its peak. The inspiratory pause is then maintained for a considerable length of time finally being terminated by an abrupt, very rapid, expiration. The apneustic curves of hypercapnia (fig. 3 G, H, I) present almost the exact opposite sort of picture, inspiration proceeding more rapidly than normal and expiration more slowly. The only common attribute of cooling and CO_2 curves is the presence of maintained inspiration. Yet it was the production of apneuses by CO_2 that led Barcroft to state that "carbon dioxide acted like making suitable cuts

through the medulla," and doubtless confirmed his belief in the predominantly depressant action of CO_2. The records of Barcroft and Margaria (1932) also show this increased speed of inspiration and Barcroft (1934) speaks of apneusis as being characterized by increased speed of inspiration; Taylor's records of apneusis produced by cyanide show both types of apneusis.

Any mechanism that slowly and gradually builds up to a maximum and then by virtue of continued supply of energy and lack of a restraining influence continues to discharge, will produce the type of picture presented by cold apneusis. But conversely, where energy input is very abrupt and rapid and continues at a high level, a sudden inspiration, and very long delayed expiration will result. Expressed in terms of C. E. S. and C. I. S. these illustrations fit into the scheme laid down by Gesell and co-workers (1937). We have here, then, a method of evaluating the mode of action of various respiratory procedures. It is not conceivable that rapid inspiration, maintained inspiration and delayed expiration are all the results of different types of action when they are observed in one respiratory cycle. The close dependence of all the phases of the inspiratory stress period on the discharge of the inspiratory side of the respiratory mechanism is thus all the more apparent.

While at the present not desirous of entering the field of architectural organization of the respiratory center or centers, certain contradictions should be pointed out. Lumsden's and Barcroft's views as to the phylogenetic nature of the various centers (either as a group or as a single center with various degrees of inhibition) are based in the former case on the presence of apneustic respiration in chelonians and in the latter case, in part, on presence of "gasping" respiration in the hibernating marmot. Yet, Popoff and Romanovskaya (1930) were able to convert pneumotaxic to apneustic respiration in the frog by cocainization of the medulla (see Nicholson and Sobin, 1938) and Marckwald (1888) produced apneusis in the sleeping marmot by simply cutting the vagi.

SUMMARY

Carbon dioxide administered intratracheally to anesthetized dogs commonly has little effect upon respiratory rate although either slowing or accleration may occur.

Irrigation of the floor of the fourth ventricle with Locke's solution equilibrated with CO_2 may also alter respiratory rate in any direction though the usual effect is a slowing, sometimes extreme in degree. The direction of the change in rate is independent of the tension of the gas. The slowing is not prevented by vagotomy, but is diminished by cooling the floor of the fourth ventricle. Simultaneous administration of intratracheal carbon dioxide accentuates the slowing.

In order for an *extreme* acceleration of respiration to occur from any cause, the following changes must take place:

1, the amplitude of respiration must decrease.

2, the speed with which inspiration occurs must be increased.

3, the speed with which expiration occurs must be increased.

4, the duration of the expiratory pause must be shortened.

Since carbon dioxide ordinarily increases the amplitude of respiration, this factor alone tends to prevent an increase in rate. However, an increase in rate might still occur if the speed with which inspiration and expiration proceeded were increased. Vagal proprioceptive reflexes seem to be an important factor not only in interrupting inspiration, but also in increasing the speed of inspiration. Depression of these reflexes by carbon dioxide then tends to decrease the speed of inspiration and produce the type of breathing seen in vagotomy. Nevertheless carbon dioxide appears to greatly augment inspiratory activity and accelerate inspiration, so that in spite of the vagotomy effect the speed of inspiration may be increased. However, this stimulation of inspiration seems to show itself also in a persistence of inspiratory activity into expiration, thus decreasing the speed with which expiration is carried out. Occasionally, this increased inspiratory activity also results in the production of a pause at the height of inspiration—an apneustic type of breathing—another factor tending to prevent an increase in respiratory rate. In addition, the expiratory pause not infrequently is lengthened due possibly to augmentation of the activity of some mechanism superficially located in the floor of the fourth ventricle, whose normal function is that of interrupting inspiration. This is doubtless the same mechanism, moderate depression of which by cooling will completely abolish the expiratory pause, severe depression of which will lead to apneustic breathing, and marked stimulation of which, as by nicotine (Nicholson and Sobin, 1938), will cause apnea. Stimulation of this mechanism is apparently largely responsible for the marked degrees of slowing occasionally resulting from intratracheal administration of carbon dioxide and frequently resulting from irrigation of the floor of the fourth ventricle with Locke's solution containing carbon dioxide. In these cases the slowing is largely due to an increase in the duration of the expiratory pause.

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THE ACTION POTENTIALS OF VISCERAL SMOOTH MUSCLE

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Under suitable conditions visceral smooth muscles give, on electric stimulation, all or none conducted responses like single nerve or muscle fibers (Bozler, 1938). Evidence has been presented that conduction in these muscles is muscular and is due to protoplasmic continuity between the muscle fibers. The experiments which will be reported here give further support to this conclusion. They show that conduction in visceral muscles is accompanied by action potentials which differ only quantitatively from those of nerve and striated muscle, a finding which is unintelligible if the smooth muscle fibers, which are usually less than 0.1 mm. long, are considered as independent units. Additional observations will be described which throw light on the nature of the normal motility of the viscera.

There are relatively few previous reports on the action potentials of visceral muscles and of smooth muscles in general, and the interpretation of the results obtained has been rather controversial. I shall mention only the literature on visceral muscles.

Orbeli and v. Brücke (1910) recorded the action potentials of the ureter of the dog in situ. Their complicated results were tentatively explained on the assumption that the monophasic potential consisted of three waves, beginning with a positive phase. Tschermak (1919) and Funke (1921) observed a slow potential change during each contraction of the frog's stomach. According to Alvarez and his collaborators (see Berkson, 1933, for references) the pendular movements of the small intestine of the rabbit likewise are accompanied by slow potential variations. In contrast to these observations, several electric variations were found during each contraction by Foà (1914), Greene (1928) and Rosenblueth, Leese and Lambert (1933) in the uterus and by v. Brücke (1913) in the retractor penis of the dog.

These studies have not definitely established the nature of the action potentials of smooth muscle and their relation to conduction. It seems certain that the complicated, and frequently variable, results previously obtained were due to unfavorable experimental conditions. In the experiments which will be described here, thin and long muscle preparations

were used, making possible an analysis of the electric changes like that which has been given for nerve.

METHOD. Uterine and intestinal strips and the ureter of several species (cat, rabbit, guinea pig and rat) were used. The strips were cut longitudinally and were 0.5 to 1 mm. in width. The mucous membrane, and if possible also the layer of circular muscle fibers, were removed, so that the preparations consisted essentially of longitudinal muscle. For reasons previously mentioned (Bozler, 1938) uterine preparations were taken from animals during pregnancy or estrus, the latter being either spontaneous or produced by injections of theelin.

The preparations were kept in the refrigerator for 30 minutes or longer, immersed in Ringer's solution containing 0.9 per cent NaCl, 0.015 per cent $CaCl_2$, 0.042 per cent KCl and 0.02 per cent Na_2HPO_4. In the experiments with intestinal strips the concentration of KCl was frequently increased to 0.05 per cent thereby increasing the motility of the muscle.

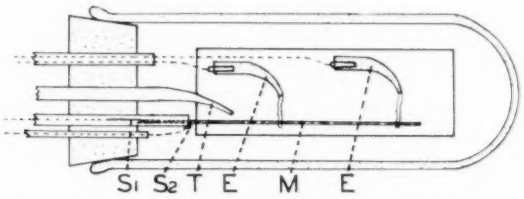

Fig. 1. Diagram of muscle chamber. E, non-polarizable electrodes; S_1 and S_2, stimulating electrodes; M, muscle; T, glass tube.

The preparations were mounted in a moist chamber (fig. 1). One end was drawn into a glass tube by means of a silver wire (S_1) serving as one of the stimulating electrodes. The second electrode (S_2) was attached to the opening of the glass tube and made contact with the tissue by a cotton wick soaked in Ringer's solution. Action potentials were led off by means of zinc-zinc sulfate electrodes, E. Solutions could be dripped directly on the preparation through the glass tube T. The chamber was immersed in a constant temperature bath. Condenser discharges ($7\mu F$) were used as stimuli.

An ink-writing recorder activated by a resistance-capacity coupled amplifier (time constant 2 sec.) was used. Subjective observation on the screen of a cathode ray oscillograph indicated that no important details of the action potentials escaped attention because of the relatively low natural frequency of the ink-writer.

The muscle preparations were suspended in air and very thin, flexible cotton wicks soaked in saline made connection with the non-polarizable electrodes. Under these conditions movements produced by stretching

usually caused only a slow shift of the baseline amounting to less than 0.05 mVolt. Because the action potentials had, in most cases, a magnitude of about 1 mVolt (rarely as much as 4 mVolts) movement artifacts were generally negligible.

RESULTS. A. *Responses to electric stimuli.* Smooth muscle has a greater tendency for repetitive discharge than nerve and striated muscle The only preparation where single shocks consistently elicited single impulses were uterine strips from cats which had received injections of theelin 3.5 to 4 days preceding the experiment (2 injections of 500 int. units at two day intervals), a period which is slightly shorter than that required for producing full estrus.

Figure 2 is an example of the potentials of such a preparation. The diphasic potential begins with a variation indicating negativity of the

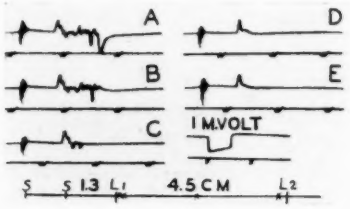

Fig. 2. Action potentials from a strip of the cat's uterus. Theelin injections 4 days before the experiment. Upward movement indicates negativity of the first lead. First deflection is stimulus artifact. Below diagram of set-up. *S*, stimulating electrodes; L_1 and L_2, leads to amplifier. First deflection is stimulus artifact. A, diphasic action potential; B, obtained after pinching 2 mm. from L_2; C, after pinching 2 cm. from L_1; D, after pinching 2 mm. from L_1; E, after pinching 1 mm. from L_1. Calibration 1 mVolt. Time marks every second. Temp. 34°.

first lead and ends with a brief spike in the opposite direction. The remainder of the record is irregular, giving the impression of a repetitive discharge. However the irregularities are due to "extrinsic effects" as can be shown by blocking conduction between the leads. By placing an injury closer and closer to the first lead a purely monophasic potential can be approached. To obtain a sufficiently undistorted monophasic potential the injured region must be closer to the lead than in nerve (1–2 mm.). The reason for this difference is the slowness of conduction as compared with the time relations of the electric response (see Bishop, Erlanger and Gasser, 1926, for a discussion of the conditions of obtaining monophasic potentials). Local injury was produced by pinching. In most cases the action potentials gradually became diphasic again a few minutes after this procedure.

Diphasic potentials obtained by two leads at a considerable distance

are often useful because the electric changes at the first lead are over when the impulse reaches the second lead. Consequently the diphasic wave probably is an undistorted record of the potential change near the second lead. The duration of this wave agrees well with that of the monophasic potential (about 0.2 sec. at body temperature).

The size of the spike potential can be varied by the action of drugs. In the cat's uterus, adrenaline ($1:10^6$ dripped on a thin strip) diminishes the electric excitability, rate of conduction and the height of the spike potential; in higher concentrations it abolishes excitability and blocks conduction (fig. 3). Cocain (1:10,000) has the opposite effects and, at higher concentrations, produces spontaneous discharges.

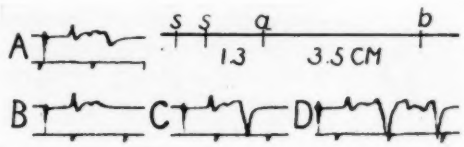

Fig. 3. Action potentials from a strip of the cat's uterus. Theelin injections 4 days before the experiment. *S*, stimulating electrodes; *a* and *b*, leads to amplifier. A, diphasic action potential; B, adrenalin 1:200,000 applied on *b*. C, cocain 1:10,000 applied on *b*; D, several minutes after C. Temp. 34°.

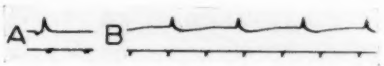

Fig. 4. Monophasic action potentials from intestinal muscle. A, strip of the smal intestine of the rabbit. Single impulse elicited by electric shock. The strip was pinched 1 mm. from the first lead. Temp. 35°. B, strip from a tenia of the colon of the guinea pig. Tonic contraction. Conduction blocked 2 mm. from the first lead. Temp. 37°.

Essentially the same potentials were recorded from the other visceral muscles used (fig. 4). These preparations, however, usually discharged repetitively on any kind of stimulation. In the experiments on intestinal muscle a further difficulty resulted from the frequent spontaneous contractions which did not always permit an impulse to be conducted over a sufficiently long distance. Strips from the small intestine of the cat and from the teniae of the colon of the guinea pig frequently became quiescent, while remaining excitable, some time after they were mounted in the moist chamber. They responded under these conditions essentially like the uterine preparation just described. The duration of the monophasic action potential and the rate of conduction varied considerably, probably depending on the degree of injury.

The duration of the spike potential may be appreciably shorter than

that of the monophasic potential because the latter perhaps includes after-potentials and may also be influenced by the width of the leads. Judging from the maximal frequency of discharge of fresh preparations the duration of the spike potential must be less than 0.2 second in the uterus of the guinea pig, less than 0.015 second in the small intestine of the cat and rabbit at 37° and less than 0.06 second in the ureter of the guinea-pig at 30°. A comparison of the mechanical and electrical responses shows that there is no detectable electric change during relaxation.

The results just described show conclusively that the passage of a wave of excitation in visceral muscles is accompanied by a potential change which differs from that in striated muscle and nerve only by a time factor. The agreement between the different types of tissues is significant in view of the great differences of structure and confirms the previous conclusion that visceral muscles are, physiologically, the equivalent of single large muscle fibers. These findings are intelligible only if syncytial connections between the muscle fibers are assumed.

B. *Spontaneous activity.* The rhythmic movements of visceral muscles are accompanied by bursts of impulses. This is true also for organs where only a single potential change during each contraction had been reported. The ureter of most species is an exception which, however, is more apparent than real, as will be shown later.

The frequency and duration of the discharge during a single contraction vary to some extent under different conditions but they are fairly characteristic for different types of muscle. In uterine muscle the frequency is relatively low, 1 to 4.5 per second; the discharge lasts for 4 to 60 seconds and ends abruptly. The duration of a burst is greater in animals at late pregnancy than during estrus. The discharge is relatively irregular if the frequency is low, as in figure 5A, but may be very regular, as illustrated by figure 5B.

The bursts of impulses which accompany the pendular movements of the rabbit's and cat's small intestine (fig. 6) are brief; the impulses gradually decline in height during each burst and have a greater frequency than in the uterus. On stretching, bursts lasting for 10 to 20 seconds can sometimes be elicited; they may be related to the peristaltic movements. After a preparation has been stretched appreciably a slow tonic discharge may occur, the frequency of which is increased by further stretching (fig. 6 D).

The teniae of the colon of the guinea pig become active only if fairly well stretched. The discharge is almost continuous and may last for half an hour with gradually decreasing frequency (fig. 4B), but periodic changes of frequency and of the size of the impulses often occur. Stretching during a period of activity increases the frequency of impulses.

It is well known that stretching increases a tonic contraction or may elicit a response in smooth muscle. This behavior is in accordance with

the experiences on action potentials just described. Although mechanical and electric responses have not been recorded simultaneously it seems certain that the frequency of discharge is an important factor determining the strength of the mechanical response as in skeletal muscle.

Intestinal strips frequently become quiescent some time after they have been mounted. Acetylcholine applied to such a preparation elicits an irregular and almost continuous discharge. This observation is analogous to the effect of adrenaline on the nictitating membrane of the cat observed by Eccles and Magladery (1937).

From the observations just described it may be concluded that the automatic contractions of visceral muscles are tetani.

Two additional points regarding the responses of smooth muscle are of general significance:

a. The frequency and duration of a burst of impulses is not influenced by the strength of the electric stimulus. It has been shown previously (1938) that the mechanical response produced by a single conducted im-

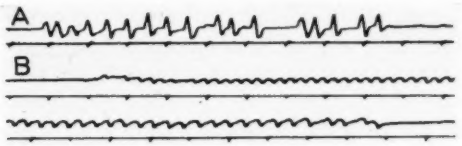

Fig. 5. Potentials from uterine strip of the guinea pig during estrus. A, brief discharge, diphasic, distance of leads about 3 mm. B, discharge of long duration. Distance of leads 4.5 cm., but activity only in the region of one lead. Impulses distorted by diphasic artifacts. Time marks every second. Temp. 37°.

pulse follows the all or none relation. This relation also holds for a whole burst of impulses.

b. In uterine preparations, where spontaneous activity usually occurs at fairly long intervals, it can be shown that the electric excitability is greatly diminished following a burst of impulses. A second burst usually cannot be elicited for 10–20 seconds, sometimes a minute after the previous one, and one to several minutes are required for a complete restoration of the previous excitability. The responses elicited during the period of decreased excitability consist of fewer impulses discharged at slower frequency than those evoked during a period of maximal excitability.

It seems probable that the decrease of excitability following a burst of impulses is analogous to the subnormal period of nerve with the only difference that the duration and extent of the depression is much greater. There is also some evidence that a burst is followed by a positive after-potential, but this point requires further investigation by means of a battery coupled amplifier.

The existence of a subnormal period helps to explain some features of the spontaneous discharge of smooth muscle. It may be assumed that the excitability gradually decreases during each burst due to the accumulation of subnormality. This change probably causes the gradual decline of frequency and the final cessation of the discharge (see particularly figs. 5B and 7).

C. *Ureter.* The action potentials of the ureter offer some interesting peculiarities and will, therefore, be considered separately. Among the four species tested the guinea pig is unique because only in this animal the

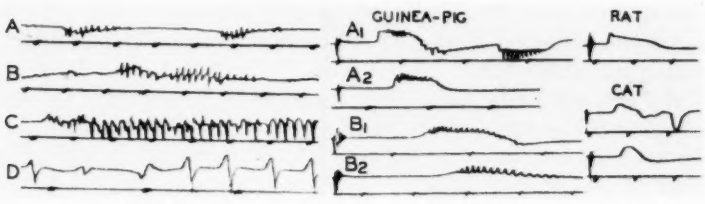

Fig. 6 Fig. 7 Fig. 8

Fig. 6. Action potentials from strips of the rabbit's small intestine. Diphasic, distance of the leads 3 mm. A and B, pendular movements. C, discharge lasting for 14 seconds, produced by stretch; only first part of response is shown. D, tonic discharge. The preparation was stretched about 5 per cent of its length while the record was taken, producing an increase of the frequency of impulses. Time marks every second. Temp. 37°.

Fig. 7. Action potentials of the guinea pig's ureter elicited by electric shocks. A_1 and A_2, diphasic and monophasic potentials of the same preparation. Distance of the leads 2.2 cm. A_2, obtained after pinching 2 mm. from the first lead. B_1 and B_2, monophasic potentials from another preparation. B_2, response during subnormal phase, 12 seconds after B_1. The frequency and duration of the discharge in B_2 are diminished and conduction slowed. Note the difference of time scale in B_1 and B_2. Time marks every second. Temp. 30.5°.

Fig. 8. Action potentials of the ureter. Diphasic potential for the cat (distance of leads 3.5 cm.); monophasic potentials for the rat and cat, obtained by pinching the preparation 1 mm. from the first lead. Time marks every second. Temp. 34° for the rat's ureter, 32° for the cat's ureter.

passage of a peristaltic wave of the ureter is accompanied by a repetitive discharge, whereas a single slow negative variation occurs in other species (figs. 7 and 8). In the guinea pig the electric responses resemble those of other visceral muscles except that the rate of discharge is surprisingly high. At the beginning of a burst, the frequency is about 7 per second at 20°, 18 per second at 30° and probably about 40 per second at body temperature. As in uterine muscle, the discharge consists of fewer impulses and has a lower frequency during the subnormal period than during a period of maximal excitability (fig. 7B). The entire burst follows the all

or none relation. One never obtains less than about 15 impulses by a single stimulus.

It can be computed that the distance between two successive impulses along the ureter of the guinea-pig is only 2 mm. Very small leads are, therefore, necessary for clearly separating the individual impulses.

It came as a surprise that no evidence of a repetitive discharge was found in the ureter of the cat, rabbit and rat. Also on the screen of the cathode ray oscillograph the potentials of these organs appeared as single smooth elevations. To explain this peculiarity it seemed possible that the electric response of the muscle fibers was repetitive and that the spikes were blurred because of the asynchronous activity of different strands of muscle fibers within a cross-section of a preparation. However it is difficult to believe that lack of synchronism would smooth out the records so that no trace of rhythmic activity could be found, even at high amplifications. Furthermore the ureter of the rat, which is much thinner than that of the guinea pig, also gave perfectly smooth potentials.

It is significant that the monophasic potential of the ureter of the cat, rabbit and rat last much longer than single impulses in other smooth muscles. The ureter of the rat is particularly interesting because its potential has a plateau lasting for 1.5 to 3.5 seconds and has, in this respect, a striking resemblance to the monophasic potential of cardiac muscle (fig. 8). At the beginning of the records there is a spike which is smaller and briefer, the closer the injured region to the lead and which is caused by a diphasic artifact. Diphasic potentials show typical "R" and "T" waves which can be readily explained as the difference between the potential changes at the two leads.

Monophasic and diphasic potentials were recorded for comparison from thin strips of the heart of turtles. The records obtained were almost indistinguishable from those of the rat's ureter.

Previous studies (Bozler, 1938) indicated that the musculature of the ureter differs from that of the uterus in the same manner as cardiac muscle differs from skeletal muscle. The peculiarities, common to cardiac muscle and the ureter, are the long "refractory phase" and the lack of any summation of the mechanical responses. It is interesting that this similarity is reflected also in the action potential.

The long continued negativity of the rat's ureter can hardly be considered as analogous to the brief single impulses of other smooth muscles. In view of the repetitive character of the discharge in the guinea pig's ureter the potentials of the ureter of other species may be regarded as the equivalent of a burst of impulses which have fused into a continuous excitatory state. The long period of depressed excitability, which had previously been called refractory phase, then, corresponds to the long, subnormal period following a spontaneous contraction in other visceral

muscles. Furthermore the absence of summation of the mechanical response appears intelligible since each peristaltic wave already is essentially a tetanic contraction. This viewpoint brings the properties of the ureter in line with those of other smooth muscles.

SUMMARY

Thin strips of intestinal and uterine muscle and of the ureter were studied. The monophasic potentials consist of brief negative variations. Diphasic potentials are complicated by "extrinsic" effects. The electric changes during a conducted response differ only quantitatively from those observed in nerve and striated muscle. An explanation for the similarity of the action potentials of these tissues is provided by the assumption of protoplasmic continuity between the smooth muscle fibers.

Each spontaneous contraction of visceral muscles is accompanied by a burst of impulses. The rate of discharge varies within wide limits; it may be as high as 40 impulses per second. A continuous discharge occurs during tonic contractions.

The monophasic potential of the ureter of most species consists of a single elevation, lasting longer than single impulses in other smooth muscles and resembling the monophasic potentials of cardiac muscle. Because, in the guinea pig, a repetitive discharge occurs during each response, the long continued state of negativity in the ureter of other species may be regarded as the equivalent of a burst of impulses. This view explains the apparent differences between the properties of the ureter and other types of smooth muscle.

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HEMOGLOBIN STUDIES ON THE BLOOD OF FEMALE MICE OF THE CBA STRAIN: EFFECTS OF AGE, DIET, STRAIN, AND REPRODUCTION[1]

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A striking paucity of information on the subject of mouse blood is evident from Scarborough's (1930–31) exhaustive review of the literature dealing with the blood of mice. Although rats are still employed more frequently than mice in many investigations, the high cost of substances used in experimental work, such as amino acids and other pure chemical compounds, often makes the choice of the smaller animal imperative. McClendon and Street (1935) have suggested, indeed, that the use of inbred strains of mice to replace rats in such studies would result in saving thousands of dollars. Hence enlarging our scant sum of knowledge about the physiology of the mouse will be of value to all the varied groups of research workers who are using these animals.

METHOD. The mice reported in this study belong to the CBA strain (Strong, 1936). They are relatively homozygous and have been bred for longevity over a period of many years.

The ages at which samples of blood were obtained from the mice are given in tables 1 and 2. Some of the animals were used at two or more of the ages indicated, but adequate rest periods of 40 days prevented the development of anemia.

For several generations the progenitors of the mice and the animals themselves from birth and also throughout the experimental period, had been maintained on a diet of fox chow.[2] One group of mice (Strong and Francis, 1937) used at 400 and 440 days of age and similarly reared, were restricted to a stock ration consisting of an oatmeal mixture.[3]

[1] Aided by grants from the Josiah Macy, Jr., Foundation.

[2] Fox chow is a commercial food supplied by the Ralston Purina Company, St. Louis, Mo. They state it "is a combination of dried meats, dried milk, cereals, vegetable matter and other concentrates."

[3] The oatmeal mixture used by Strong for several years has the following composition: rolled oats (Pratt's or Avena) 82.3 per cent; "50 per cent protein meat scrap" (Baugh and Son, Philadelphia, Pa.) 5.8 per cent; powdered whole milk 10.3 per cent; sodium chloride 1.6 per cent.

Litters were weaned at 30 days of age and thereafter the mothers were "rested" for several days before serving as subjects for this study. It is not known whether mice are subject to a temporary anemia associated with gestation and lactation as are the rat and man (Mitchell and Miller, 1931), but under the conditions of this experiment it would be highly improbable that the concentration of hemoglobin in the blood of the breeders used would be other than normal. Furthermore, in only a few instances were mice employed which had cast litters as recently as 40 days before furnishing blood.

The animals were separated from food for 16 to 20 hours prior to the withdrawal of one sample of blood each at the ages indicated. While a mouse rested comfortably in a specially designed glass holder (Francis, 1936) venous blood was taken from the tail through a small puncture made with a pointed curved blade. Accuracy in measuring and in diluting the blood was assured through the aid of a magnifying lens and by the use of Newcomer pipettes calibrated by an improved technic (Francis, 1937). The acid hematin method was used and readings were made colorimetrically, with all conditions controlled (Strong and Francis, 1937) which might possibly affect the exactness of the readings.

In order to be assured of the healthy condition of the mice upon whose blood hemoglobin had been determined, four months after the completion of the studies an examination was made of the history of each animal for the period subsequent to that of the last withdrawal of blood. It is well known that the appearance of a mouse or the body weight frequently is not a good criterion of its physical state. Moreover, with the approach of death from any cause, it was assumed that mice would be in such physical condition that their hemoglobin concentration might be abnormal.

By careful inbreeding this strain of mice has acquired a high resistance to spontaneous tumors but is not completely immune, hence an examination was made of a large number of data pertaining to the onset and development of spontaneous mammary tumors in another strain of susceptible mice. By extrapolation the origin of such abnormal growths was calculated (Strong) to be from 4 to 6 weeks before detection by palpation became possible. The hemoglobin records of each mouse which within 80 days of furnishing blood had developed a tumor, or in which one was first observed at autopsy, were then removed from each list. A period of 80 days was considered sufficient to insure absence in the mouse of conditions, as yet unknown, which doubtless presage the onset of such neoplastic growths. Hemoglobin records of those mice which, within a 40 day interim, had died from any other disease were likewise deleted from each list.

Among the 9 groups which were reduced, the average values for hemoglobin in 6 of the deleted records were identical with those of their corre-

sponding lists in the unreduced forms, even though one group of 38 determinations was shortened by as many as 6, and another of 29 by 5. Each of the remaining 3 deleted lists furnished an average which varied by only a fraction from the average figure for hemoglobin of the respective unaltered group of records. The significance ratios calculated for these 3 groups showed that the slight variations obtained were far from being significant. Furthermore, the average values for hemoglobin were not altered by a trial removal of a few determinations from any of the remaining large groups; hence, under the conditions of this investigation, averages presented for all lists composed of a large number of determinations can be considered truly representative of the level of hemoglobin for the strain of mice used. The average values reported might be questioned only in instances where groups were very small.

Some individual hemoglobin records deviated widely from the averages for the respective groups of determinations, even though the mice were highly inbred and experimental conditions were the same for all animals. Similar variations in hemoglobin levels in the blood of Swiss and Rockefeller mice have been observed by De Kock (1931). The average values for hemoglobin presented in tables 1 and 2 have been derived from determinations made upon samples of blood taken from 687 breeder and 142 virgin mice.

Results and discussion. The data for breeders indicate that a maximum value of 16.3 grams per 100 cc. of blood occurred at 200 days of age; during the following 160 days the drop in hemoglobin amounted to only 0.7 gram per 100 cc. of blood; for the subsequent 120 days, or up to 480 days of age, the hemoglobin was lessened by 1.2 grams; thereafter up to 720 days of life, covering a span of 120 days, the fall in hemoglobin amounted to only 0.8 gram per 100 cc. of blood. Thus the average values for hemoglobin, within narrow limits, describe two plateaus separated by a period of 120 days during which the blood was depleted of an appreciable amount of pigment. The erratic trend of values subsequent to 720 days of life is due in all probability to the small number of determinations constituting each of the last 5 groups.

The concentration of hemoglobin in the blood thus varies with age in mice as it does in other animals. The effect of age in the rat has been reported by Williamson and Ets (1926), Sure, Kik and Walker (1929), Mitchell and Miller (1931), Orten and Smith (1934), and by many others, most of whom, however, used only a few animals at each of the ages studied. Williamson (1916) has reported the effects of age upon the levels of hemoglobin in man.

An average of 19 to 20 grams of hemoglobin per 100 cc. of blood has been reported by De Kock (1931) for 1400 Rockefeller and Swiss female mice which, except for variations in diets, were studied under uniform

experimental conditions. Hemoglobin values for 21 per cent of these mice reached 20 grams, while only 8 per cent gave as low a value as 17 grams, and still fewer had concentrations less than 17 grams per 100 cc.

TABLE 1

*The hemoglobin concentration in the blood of breeder (B) and virgin (V) mice of the CBA strain maintained on a diet of "Fox Chow"**

AGE OF MICE	NUMBER OF DETERMINATIONS		HEMOGLOBIN IN GRAMS PER 100 ML. OF BLOOD						
			Group averages		Significance ratio	Deviations from averages			
						Above		Below	
	B	V	B	V		B	V	B	V
days									
40		30		16.0			1.9		2.4
80		26		16.9			2.0		1.8
120	5	4	15.9	17.4	3.9	0.8	1.0	0.7	1.3
160	19	6	16.2	16.9	4.0	1.4	0.4	1.7	0.7
200	37	6	16.3	16.6	0.8	2.4	2.1	1.7	1.5
240	51	19	16.1	15.8	1.7	2.6	2.4	2.0	1.6
280	55	20	15.9	15.3	3.7	4.5	2.1	2.0	1.1
320	51	16	15.6	14.5	7.0	1.8	1.8	2.4	0.9
360	61		15.7			2.0		1.5	
400	64	6	15.3	15.2	0.4	2.1	0.8	3.1	0.8
440	51	6	14.9	14.6	1.8	2.9	0.4	4.6	0.7
480	33	3	14.5	13.9	0.9	1.8	1.1	3.0	1.9
520	40		14.5			1.4		3.1	
560	38		14.5			2.3		2.7	
600	33		14.2			1.5		1.1	
640	29		14.2			2.6		3.4	
680	17		13.7			1.3		2.4	
720	12		13.7			1.6		1.5	
760	8		13.2			2.0		1.9	
800	4		13.0			0.3		0.2	
840	2		13.5			0.7		0.7	
880	2		13.5			0.05		0.05	
920	2		12.7			0.9		0.8	

* Significance ratio $= \frac{D}{PEd}$, where D is the difference between two averages, PE is the probable error, and

$$PEd = \sqrt{(PE \text{ of one average})^2 + (PE \text{ of the other average})^2}$$

The method of calculation used requires a ratio of 3 or over for a difference to be mathematically significant.

of blood. The hemoglobin values of these two "fairly well standardized" strains of mice thus are much higher than the concentrations reported in this study for another strain of standardized mice. It is not possible to explain fully the wide variations in blood pigment among mice of the same

strain (tables 1 and 2), and though the disparity between these data and De Kock's may be due in part to dissimilarity in the diets employed in the two laboratories, nevertheless Strong and Francis (1937) have shown that under uniform experimental conditions there is a significant difference in the blood of two standardized strains of mice. A knowledge of the influence of intrinsic as well as extrinsic factors in modifying the blood picture of mice, serves to emphasize the need for caution in attributing to abnormal states, concentrations of hemoglobin in mice falling within a range of about 12 to 20 grams per 100 cc. of blood.

In table 2 are shown the effects of different stock rations upon the hemoglobin levels in a single strain of mice. The trend of these values is in line with the observations of Bittner who reported the superiority of fox chow over his oatmeal mixture, with reference to the reproductive behavior of an inbred strain of mice.

TABLE 2

The effect of diet on the hemoglobin concentration in the blood of breeder mice of the CBA strain

AGE OF MICE	NUMBER OF DETERMINATIONS		HEMOGLOBIN IN GRAMS PER 100 ML. OF BLOOD						
			Group averages		Significance ratio	Deviations from averages			
						Above		Below	
	B-F	B-O	B-F	B-O		B-F	B-O	B-F	B-O
days									
400	64	51	15.3	14.6	3.8	2.1	7.1	3.1	3.9
440	51	22	14.9	14.3	2.7	2.9	1.6	4.6	3.5

* "B-F" mice were restricted to a diet of "Fox Chow" and "B-O" mice to a ration consisting of an oatmeal mixture. Details of diets appear on p. 511.

Diet is probably the most important extrinsic factor capable of changing the amount of hemoglobin in the blood of animals. Mitchell (1932) found it necessary to prevent both coprophagy and ingestion of minerals which rats easily obtain from the ordinary metal cage and food cup, because such small additions to her experimental diet seriously interfered with the production of nutritional anemia in rats. Moreover, evidence of dissimilarity in the concentration of hemoglobin in the blood of the albino rat raised upon different stock diets, is furnished by Mitchell and Miller, (1931), Orten and Smith, (1934), and other workers.

The hemoglobin determinations on the blood of virgin mice at 40, 80, 240, 280, and 320 days of age represent for each group a sufficiently large number of determinations for the average figures to be reasonably valid. Unfortunately lack of funds prevented the logical completion of this study. From the data available one can only speculate whether, in animals of

the same strain and with all conditions carefully controlled, the amount of hemoglobin in the blood of virgin mice may be significantly different from that of breeders.

SUMMARY

Hemoglobin values have been determined on the blood of 829 normal inbred mice of the CBA strain at 40 day intervals, from 40 to 480 days of age in the case of virgins, and from 120 to 920 days for breeders.

A maximum average of 17.4 grams of hemoglobin per 100 cc. was obtained from the blood of 4 virgin mice when they were 120 days old, and one of 16.9 grams from the blood of 26 virgins at 80 days of age.

A maximum average of 16.3 grams of hemoglobin per 100 cc. was obtained from the blood of 37 breeders 200 days old. The average values for hemoglobin in the case of breeders describe two relatively level plateaus which average 15.9 grams for the first period of 160 days and 14.2 grams for the second interval of 240 days. The plateaus are separated by an interim of 120 days during which the hemoglobin was lowered by 1.2 grams. Hence there is a gradual loss of blood pigment associated with age.

Feeding fox chow for several generations gave higher levels of hemoglobin than was found in the same strain of mice maintained similarly on a ration consisting of an oatmeal mixture.

Age, diet, and strain should each be taken into account in interpreting data dealing with concentrations of hemoglobin in the blood of mice.

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AN ANALYSIS OF THE BASAL METABOLISM, BODY TEMPERATURE, PULSE RATE AND RESPIRATORY RATE OF A GROUP OF PUREBRED DOGS

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The following study was carried out in order to secure a set of well-established data on the basal metabolism, body temperature, pulse rate and respiratory rate of the normal dog. Such data would be useful in evaluating experimental results.

The dogs selected were bull terriers. Since the stock was purebred, variations in glandular structure were probably at a minimum. A mixture of cooked rice and meat with chopped raw lettuce, milk, calcium carbonate, sodium chloride and lactose was fed daily at 11:00 a.m. Cod-liver oil was given except during the summer. In addition, each animal was given two dog biscuits except on the evenings preceding the days on which metabolism determinations were to be made. This diet seemed quite adequate and the dogs were in a good state of nutrition according to Cowgill and Drabkin's (1) chart. The animals were kept outside in pens large enough for ample exercise. Except in summer, the dogs slept indoors at night. At semi-weekly intervals, from the age of three months on, the dogs were trained to lie quietly, without restraint, on their right sides. The animals coöperated so well that they occasionally fell asleep. A half-hour rest period preceded each six-minute metabolic test.

A Benedict-Roth recording metabolism apparatus was used. A gas-tight seal between an adjustable rubber mask and the dog's muzzle was secured with the aid of a three per cent aqueous gum tragacanth jelly. Surface areas were calculated by means of the Cowgill and Drabkin (1) formula.

Basal metabolism determinations were regularly performed on each dog at monthly intervals for a period of about two years. Extra values were obtained during the last several months. The first tests were made on litter-mates 01, 02, 03, 04 and 05 when they were 13 months old. Dogs 10, 11 and 12 from the same parents, were 4 months old when started.

Dogs 06, 07 and 08 having the same father but a different mother, were started at the age of 9 months.

The animals were not subjected to any sort of experimental treatment except that x-ray pictures of the head, spine and right hind leg were made once a month in connection with a study of normal calcification. This work will not be reported at this time.

Discussion of results. The histogram of figure 1, based on 355 pooled observations from 11 dogs, indicates that most of the basal metabolic values are normally distributed. The following causes probably contributed to the sporadic high values. The rapid increase in the surface area of the growing young animals was associated with a higher basal metabolism as can be seen from figure 2. After 10 months of age, the surface area increased quite slowly and within the period covered by the present observations, the basal metabolism became essentially constant. Boothby and Sandiford's (2) standards for human beings, indicate the existence of a somewhat similar type of relationship. The transition point

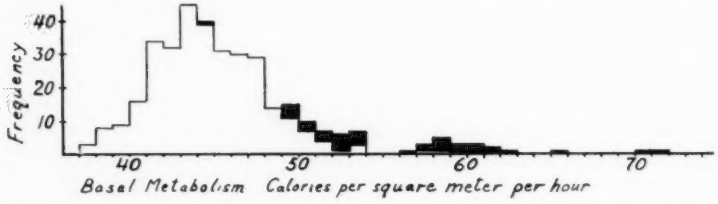

Fig. 1. Frequency distribution of basal metabolism values. The shaded portions represent the first three values obtained with each dog.

occurred at the age of about twenty years when growth practically ceased and the rate of decrease of basal metabolism with age became very small. A second factor contributing toward higher values seems to be the fact that the animals required a short time to become accustomed to the conditions of the experiment, for even fully-grown dogs gave high figures for the first two or three experiments. During August and September of 1936 several unexplainably high values were obtained which probably did not represent the basal state.

Since statistical methods of analysis are best suited to normally distributed data, only such data (about 89 per cent of the total) were used for comparative purposes.

When compared with the basal metabolic values cited in the review by Kunde and Steinhaus (3), the results of the present study fitted into the higher grouping. About half of the results obtained by Kitchen (4) are to be found at the same high level. Only approximate comparisons can be made, however, for age and breed are rarely stated and surface areas

have usually been computed with the aid of the Meeh-Rubner formula. Cowgill and Drabkin (1) found that this formula, which considers weight but no other dimension, such as length, generally leads to high results. Consequently basal metabolism figures, expressed by the aid of this formula, would tend to be low. The results of the present series, if calculated by the Meeh-Rubner formula, would be about ten per cent lower. Since bull terriers are short-haired animals they should, according to Rubner (5), have higher basal metabolic values than long-haired dogs. Stockard (6) has shown that the relative size of the thyroid gland varies widely with the breed and the purity of the breed. This factor is of considerable im-

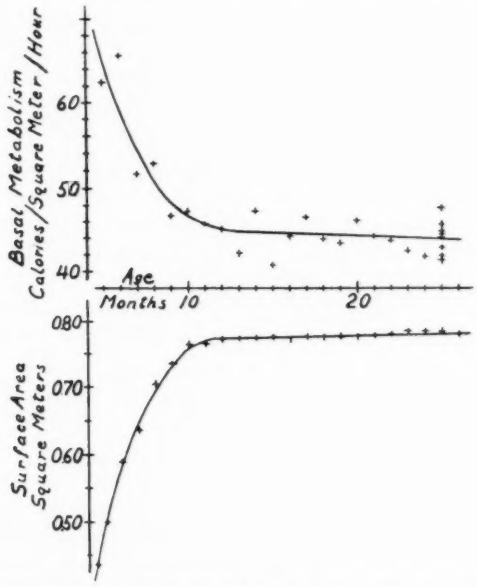

Fig. 2. The change of surface area and basal metabolism with age. Dog 11

portance and may be responsible for some of the variations in results found by different investigators.

As Boothby, Berkson and Dunn (7) observe, the lowest basal metabolic values of human beings are not necessarily the most correct ones but rather are to be regarded as the lower extreme of the distribution. The symmetry of the frequency distribution of the data for the bull terriers likewise indicates that the lower values obtained in the present study may be similarly interpreted.

A comparison of intraindividual variations and interindividual variations was made by using a simplified method, outlined by Tippett (8) which

involves the grouping of normally distributed data. Accordingly, all such observations were so treated. The mean variance of interindividual variation for the 11 dogs was 36.16 which corresponds to a standard deviation of 6.01. The mean variance of the intraindividual variations, based on more than 300 observations, is 7.31 and the mean intraindividual standard deviation is 2.70. This compares favorably with 2.83, the mean of the standard deviations given in table 1 which were obtained by the more laborious direct method in which no grouping was used. The significance of the difference between the larger mean interindividual variation and the smaller mean intraindividual was confirmed by calculating the statistic z which was found to be 0.7994 for $n_1 = 10$ and $n_2 = 298$. Since this is greater than the nearest corresponding value of 0.3702 in Fisher's (9) tables for $n_1 = 8$ and $n_2 = 60$ at the 5 per cent point, the mean interindividual variance is significantly greater than the mean intraindividual variance, and the basal metabolism of each dog is to be considered as a personal characteristic which may or may not differ from that of any other dog in the group. These factors should be of importance in designing control experiments. Berkson and Boothby (10) in an extensive statistical analysis found that human beings show greater interindividual than intraindividual variations. The standard deviations cited are about half as great as the corresponding values for dogs.

Although the basal metabolism can be considered characteristic of the individual, no evident relationship to such personal traits as disposition, activity or intelligence, as noted by an independent observer, was obvious. Lusk (11) in 1912 found that it was possible for a dog's metabolism to be maintained at a very constant level. The coefficients of variation in table 1 show that the results of the present study were also quite uniform, even over a long period of time. However, at the age of 23 months, dog 12 suddenly became an erratic subject, producing an occasional normal figure but for the most part giving values which were several times as great and obviously not basal. Too few females were available to draw any conclusions regarding the sex factor.

As is indicated by the coefficients of variation in table 1, the respiratory rate experienced the greatest relative variations and the rectal temperature the least. These values may be compared with those of Whiting (12) for human subjects: 17.80 for the respiratory rate, 14.89 for the pulse rate and 0.49 for the oral temperature. No evident relationship could be discerned between the body temperature, the pulse rate or the respiratory rate and the size of the animals in this group. The analysis of respiratory rates does not include those cases in which panting occurred. About 12 per cent were of this type. Over half of these cases were associated with perfectly normal basal rates. The remaining twenty cases, which occurred

TABLE 1

DOG	SEX	SEPTEMBER 1936				BASAL METABOLIC RATE CALORIES PER SQ. M. PER HR.				RECTAL TEMPERATURE				PULSE RATE BEATS PER MINUTE				RESPIRATORY RATE RESPIRATIONS PER MINUTE			
		Age	Length	Weight	Surface area	Number of observations	Mean	Standard deviation	Coefficient of variation	Number of observations	Mean	Standard deviation	Coefficient of variation	Number of observations	Mean	Standard deviation	Coefficient of variation	Number of observations	Mean	Standard deviation	Coefficient of variation
		mo.	*cm.*	*kgm.*	*sq. m.*						°*F.*	°*F.*									
02	♂	34	90	24.1	0.824	31	42.94	2.88	6.7	32	100.30	0.54	0.54	33	77.9	6.0	11	29	15.1	2.9	19
06	♂	30	96	25.2	0.896	32	43.67	2.74	6.3	35	100.41	0.51	0.51	35	84.4	6.8	8	23	14.6	3.7	25
05	♂	34	87	21.0	0.757	27	44.28	2.39	5.4	28	100.23	0.27	0.27	29	78.3	8.4	11	23	17.0	3.2	19
11	♂	25	88	21.5	0.773	25	44.33	2.51	5.7	28	100.55	0.47	0.47	28	77.1	10.4	14	21	15.2	2.3	15
03	♂	34	91	26.8	0.868	34	44.41	3.18	7.2	34	100.66	0.51	0.51	34	82.6	10.2	12	32	14.6	2.3	16
10	♂	25	86	22.7	0.771	24	45.04	2.61	5.8	28	100.51	0.48	0.48	29	81.7	9.8	12	25	13.1	2.7	21
07	♂	30	92	25.3	0.859	30	45.18	2.45	5.4	32	100.52	0.46	0.46	32	81.2	7.7	10	26	16.3	3.1	19
08	♂	30	90	24.1	0.824	30	45.50	3.15	6.9	30	100.42	0.48	0.48	29	80.1	7.7	10	26	17.3	3.0	17
01	♂	34	86	21.8	0.759	34	46.12	3.31	7.2	33	100.41	0.50	0.50	33	83.6	9.1	11	24	18.3	3.7	20
Means	♂		89.5	23.61	0.8148		44.607	2.802	6.29		100.446	0.469	0.469		80.76	8.46	11.0		15.72	2.98	19.0
04	♀	34	87	21.8	0.767	30	43.27	2.77	6.4	30	100.71	0.45	0.45	31	74.5	7.7	10	25	17.3	2.5	15
12	♀	25	84	20.5	0.725	18	43.89	3.12	7.1	29	101.18	0.51	0.51	23	83.9	9.0	11	27	15.8	2.7	17
Means	♀		85.5	21.15	0.7460		43.580	2.945	6.75		100.945	0.480	0.480		79.20	8.35	10.5		16.55	2.60	16.0
Means	♂ + ♀		88.8	23.16	0.8024		44.421	2.828	6.37		100.536	0.471	0.471		80.49	8.44	10.9		15.87	2.92	18.5

during the months of August and September 1936, were associated with very high metabolic values.

A study of correlation coefficients relating basal metabolism and such factors as body temperature, pulse rate and respiratory rate, again emphasizes individual peculiarities. As table 2 shows, regular associations of the above factors were not found. The existence of correlations was limited to neither high nor low basal metabolic values.

A small but definite positive correlation was found to exist between the room temperature and the rectal temperature. The significance of a correlation coefficient and standard error of 0.153 ± 0.061 for the pooled data from all the males was further established by transformation to a t value as described by Fisher (9). This was found to correspond to a probability of less than 0.01. This change of body temperature with room temperature was evidently not sufficiently great to affect the basal metabolism within the temperature range experienced (19°–31° C.) since a

TABLE 2

The correlation coefficient, r, and its standard error, S.E.

DOG	BASAL METABOLISM AND BODY TEMPERATURE		BASAL METABOLISM AND PULSE		BASAL METABOLISM AND RESPIRATORY RATE	
	r ± S.E.	P	r ± S.E.	P	r ± S.E.	P
02	0.025 ±0.189		0.378 ±0.182	0.05		
06	0.478 ±0.183	<0.01	0.413 ±0.180	0.02		
01	0.111 ±0.189		0.241 ±0.196		0.032 ±0.204	
08	0.435 ±0.186	<0.02	0.620 ±0.183	<0.01	0.336 ±0.196	

The probabilities in column P have been calculated with aid of Fisher's t formula when significant correlations have been found.

correlation coefficient and standard error of 0.016 ± 0.062 was obtained which indicates a lack of association between basal metabolism and room temperature. Rubner (5) found that, with the exception of the above range, basal metabolism was affected by the environmental temperature.

Changes in the barometric pressure produced no evident effects on basal metabolism, pulse rate, respiratory rate or body temperature.

SUMMARY

A collection of data on the basal metabolism, body temperature, pulse rate and respiratory rate of a group of purebred dogs has been made over a period of two years.

A statistical analysis of the data has been made and means, standard deviations, coefficients of variation, and correlation coefficients have been calculated.

The results indicate that although the measurements are closely grouped,

overlapping and reproducible, each individual yields characteristic results and exhibits its own peculiar relationships.

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STUDIES ON THE PHYSIOLOGICAL EFFECTS OF LEUKOTAXINE

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Previous studies by one of us have demonstrated the presence in inflammatory exudates of a factor capable of increasing capillary permeability (1). Purification of the exudate material has yielded a crystalline nitrogenous substance resembling in some of its properties a relatively simple polypeptide (2). This substance is extremely active in inducing prompt increased capillary permeability as shown by the pronounced seepage of an intravenously injected vital stain into treated cutaneous tissue. In its purified form evidence of activity may be still detected in concentration of 1 part in 100,000. Furthermore, this material manifests a chemotactic effect. Within 20 to 30 minutes following its intracutaneous inoculation, polymorphonuclear leukocytes accumulate in abundance in the lumen of small vessels. These cells at first adhere closely to the endothelial wall from which they soon migrate actively into the extra-capillary spaces (3). The chemotactic property of this active substance may be demonstrated *in vitro*. Polymorphonuclear leukocytes in a sample of exudate readily aggregate around particles of the material deposited on a slide (3). For the sake of convenience this substance has been tentatively named *leukotaxine* (4). Leukotaxine is thus able to reproduce much more rapidly than even very intense irritants (e.g. turpentine or aleuronat) the basic sequences of the inflammatory reaction, namely, the initial increased capillary permeability followed by the migration of polymorphonuclear leukocytes. Its liberation by injured tissue offers thus a reasonable explanation for the mechanism concerned with the fundamental pattern of the inflammatory reaction.

In the foregoing studies no concrete evidence has been obtained to support Lewis' view of an existing histamine-like substance in exudates or in their partially purified active fractions (5). The possibility, however, that histamine exists in exudates is not wholly dispelled by the available evidence on the presence of the permeability factor; but the primary impor-

[1] Aided by grants from the Milton Fund of Harvard University, from the William W. Wellington Memorial Research Fund of the Harvard Medical School, and from the International Cancer Research Foundation.

tance of histamine in explaining the mechanism of increased capillary permeability in inflammation is open to serious doubt. Leukotaxine does not exhibit the properties of histamine. Histamine either fails to induce an increase in the permeability of the capillaries of the rabbit, as demonstrated by the methods utilized, or else the increase, contrary to the action of leukotaxine, is limited solely to the periphery of the treated cutaneous areas. Furthermore, unlike histamine, leukotaxine fails to induce contraction of the isolated segment of the guinea-pig intestine. An additional chemical evidence that leukotaxine is not histamine is furnished by the chemical test of Zimmermann with 1 per cent cobalt nitrate and 2 N sodium hydroxide solution (6). Histamine interacts with these reagents to form a specific violet color. The active material fails completely to induce any such color reaction, yielding merely a blue color followed almost immediately by the formation of a yellowish suspended precipitate. In brief, no evidence has as yet been obtained to support the view (5) that histamine or its presumably closely related H substance is identical with the permeability factor.

The present report briefly summarizes observations on the effect of leukotaxine on vascular tension and on the relation of this substance to adenylic compounds. The depressing effect of histamine on blood pressure is well known (7). It is doubtless of interest to know whether the factor causing increased capillary permeability in inflammation is likewise capable of inducing a sharp sustained fall in blood pressure leading ultimately to a state resembling shock.

EXPERIMENTAL. The method of extracting leukotaxine from exudates has already been described in detail elsewhere (2). In its essential features the method consists of treating the cell-free exudate with pyridine. The precipitate is discarded and an equal volume of acetone is added to the supernatant phase. The bulk of the proteins are eliminated by precipitation. The acetone supernatant fractions can be placed at −20°C. for several days. The active material usually separates out (fraction A). A more effective product is obtained by evaporating *in vacuo* the acetone supernatant phase. The dried material is subjected to prolonged extraction with butyl alcohol. The superantant butyl alcohol fraction is either placed in a separatory funnel at −20°C. from which the active substance separates out within a few days (fraction C), or else it may be evaporated to dryness yielding a resinous material within which is found the characteristic doubly refractive crystalline substance (fraction C-D). The admixed tarry material can be removed by oxidizing with nitric acid followed by ammonium hydroxide. A substance of pale tan color is obtained which exhibits great activity (fraction E). Further studies on the purification and identification of leukotaxine will be reported elsewhere. Various tests indicate that this substance resembles in its properties a relatively

simple polypeptide. Its liberation is possibly the outcome of an interference with normal protein catabolism by the presence of an irritant.

The observations were all made on cats under dial anesthesia (0.7 cc/kilo weight). The anesthetic was administered intraperitoneally. Blood pressure measurements were recorded from the carotid artery by introducing a cannula connected in turn to a mercury manometer. Leukotaxine

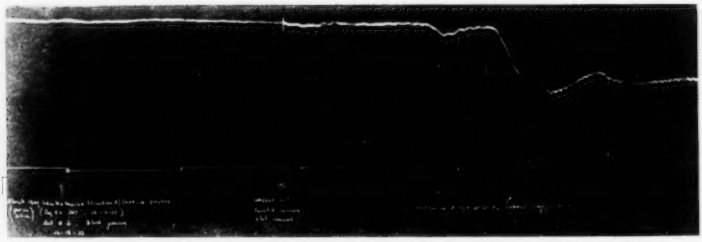

Fig. 1. Effect of leukotaxine (fraction A) on blood pressure. At the first arrow, about 19 mgm. of leukotaxine were injected intravenously. No change occurred in the blood pressure. At the third arrow, 10 mgm. of histamine dihydrochloride were injected intravenously. Note the sharp drop in pressure.

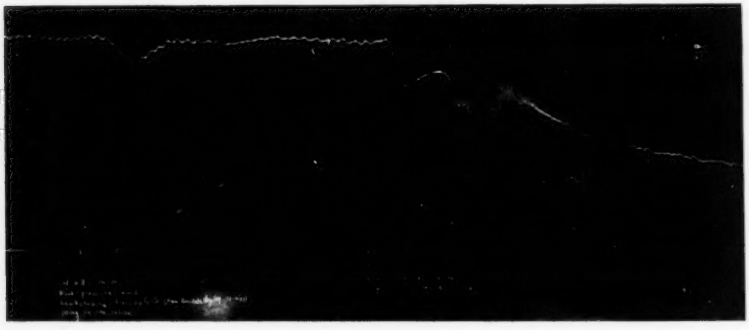

Fig. 2. The injection of 10 mgm. of leukotaxine (fraction C-D) induced a transient fall in pressure in contrast to the progressive fall elicited by histamine dihydrochloride (10 mgm.).

in doses varying from 3 to 100 mgm. in 1 cc. of physiological saline was introduced directly into the femoral vein.

Results. Fraction A or the active material which separates out from the acetone mother liquor at $-20°C$. displays no effect on the systemic blood pressure (fig. 1). This is in sharp contrast to the action of histamine which, as is well known, induces a sharp fall in tension (fig. 1). The active fraction obtained after final extraction with butyl alcohol (C-D) usually induces an immediate transient and minimal fall in blood pressure. The

original level, however, unlike histamine, is very quickly regained (fig. 2). With very low doses of leukotaxine ranging between 3 to 5 mgm. the transient fall in pressure may be either absent or barely perceptible (fig. 3).[2] Furthermore an initial injection of leukotaxine does not alter the response elicited by successive administrations of this substance. This is in sharp contrast to the action of histamine. The drop in blood pressure

Fig. 3. Three small doses of leukotaxine (3, 5, and 10 mgm. respectively) administered at successive intervals produced only a minimal depressing effect on the blood pressure which is nevertheless definite at the 10 mgm. dose. When 5 mgm. of histamine were injected intravenously a sharp drop occurred and the heart rate slowed up with an augmentation in the amplitude of its contraction.

Fig. 3a. Subsequent to a primary injection of histamine as shown in figure 3, 10 mgm. of histamine were reinjected. Note the transient increase in blood pressure level. This type of effect is not elicited by successive reinjection of leukotaxine (cf. fig. 3).

induced by the latter is frequently followed by a rise in tension upon prompt reinjection of histamine (fig. 3 and 3a). It is to be noted that leukotaxine even in doses as large as 100 mgm. administered intravenously, fails to induce a progressive fall in blood pressure which would in the remotest way suggest the development of shock (fig. 4). The introduction of large

[2] Essentially the same type of response is obtained with the further purified product (fraction E).

doses of leukotaxine reveals no conspicuous sign of increased blood viscosity or any other evidence suggesting significant reduction in plasma volume.

The question arises as to whether leukotaxine might not be related to an adenylic compound. Parnas and Ostern (8, 9) reported observations indicating a slowing of the heart following administration of adenyl triphosphate, adenylic acid, or adenosine. This finding has essentially been

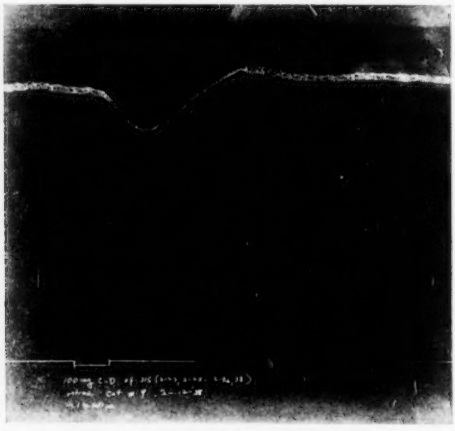

Fig. 4. The administration of a powerful dose of leukotaxine (100 mgm. of fraction C-D) fails to induce a *sustained* fall in blood pressure.

Fig. 5. Effect of leukotaxine on the heart rate. At the point indicated by the second arrow 1 mgm. of leukotaxine (fraction E) was introduced into the auricle. There is no evidence of a slowing of the heart rate. At the point indicated by the fourth arrow 1 mgm. of adenosine was introduced. The slowing of the ventricular rate indicates the manifestation of a conspicuous heart block.

substantiated by Gillespie (10). Bennet and Drury concluded that the extent and duration of the heart block in the guinea pig yielded a biological test for the adenosine equivalent of adenylic acid in a given extract (11). Adenosine induces an increase in capillary permeability as indicated by the seepage from the circulating blood of trypan blue into cutaneous areas treated with this substance. For this reason and because adenosine tends

to induce a transitory fall in blood pressure it became indispensable to determine whether leukotaxine might not be identified as an adenylic compound.

A frog was pithed and its heart exposed. The contracting ventricle was attached by a silk thread to the recording pointer of a kymograph. Leukotaxine was injected into the auricle. While the needle of the syringe was being inserted into the cardiac tissue mechanical deflections occurred on the record. Injection of leukotaxine, however, produced absolutely no change in the rhythm or rate of the heart (fig. 5). When adenosine in saline (concentration of NaCl about 0.65 per cent) was introduced into the auricle the heart rate immediately slowed up to a marked degree, thus confirming the earlier observation of the aforementioned investigators. The absence of any such effect with leukotaxine would tend to rule out of consideration an adenylic compound.[3]

CONCLUSIONS

Leukotaxine is the crystalline nitrogenous substance recovered from areas of inflammation. Its liberation offers a reasonable explanation for the mechanism of increased capillary permeability and leukocytic migration in areas of injury. When introduced into the circulating blood stream it fails to induce a sustained fall in blood pressure. In moderate or large concentration it tends to produce either no change or only a transient and practically negligible fall in vascular tension. This effect is in sharp contrast to the progressive and marked drop in level induced by similar concentrations of histamine. Observations on the heart rate of frogs indicate that leukotaxine probably bears no relation to adenylic compounds.

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[3] Acetylcholine with or without eserine fails to induce an increase in capillary permeability. Furthermore in contrast to leukotaxine (1), acetylcholine induces a contraction of the isolated loop of intestine. These facts in addition to the effect on the blood pressure tend thus to discount likewise acetylcholine as a significant factor in determining the nature of leukotaxine.

THE RELATION OF THE LIVER TO CREATINE-CREATININE METABOLISM

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At the present time it is generally believed that the liver plays no part in creatine-creatinine metabolism (1), although for some reason negative evidence on this point has always seemed to carry more weight than positive evidence. In the metabolism of these substances, however, it is necessary to consider their formation, distribution, transformation into each other (and possibly into other products), and their excretion. The liver is vitally concerned with most metabolic changes in the body and it would indeed be surprising if it did not also play a very significant rôle in creatine-creatinine metabolism. It is also well known that any condition which causes a decrease in body glycogen will result in creatinuria. This is true both in diseases of the liver or in liver damage due to phosphorus or chloroform poisoning. It is therefore evident that the liver does play some part in creatine metabolism, and it was the purpose of this study to determine what that part is.

EXPERIMENTAL. Six dogs were used as experimental animals. The technique of liver removal was the two stage procedure described by Markowitz and Soskin (2). At the first operation,[1] through a midline incision, the inferior vena cava proximal to the lumboadrenal vein was loosely ligated with stout linen, thus occluding about four-fifths of the lumen. The portal vein at the point of bifurcation in the portal fissure was similarly ligated proximal to the last tributary. The intestine became mildly congested but not cyanosed.

During the five weeks following the operation the animals were fed on Checkers (Ralston-Purina Co.) and meat was added twice a week.

A 24 hour specimen of urine was obtained from dogs 3 to 6 for 1 day before the first operation and on the same day thereafter each week following it. A 1 day urine sample was obtained from dogs 1 and 2 before the second operation for complete liver removal, and all urine was saved for the length of time the animals lived thereafter. Total nitrogen was deter-

[1] The operations in this study were all performed by Dr. Frederick Fitzherbert Boyce of the Department of Surgery of this School, who will report elsewhere his studies of the temperature reactions.

mined by the Kjeldahl procedure, using the Kirk microapparatus for distillation of the ammonia; total creatinine was determined by a slight modification of the method of Benedict and Myers (3), and preformed creatinine by alkaline picrate as usual (4). The difference between the total and preformed creatinine represents creatine, as creatinine, in chart 1.

The second operation was performed 6 weeks after the first. The ether anesthesia was so slight that the animal recovered from it very soon after the operation was complete. Through the original midline incision the structures in the lesser omentum, and the vena cava above and below the liver, were ligated and cut. The entire liver was then easily removed. Immediately after the operation was completed 10 per cent sterile glucose

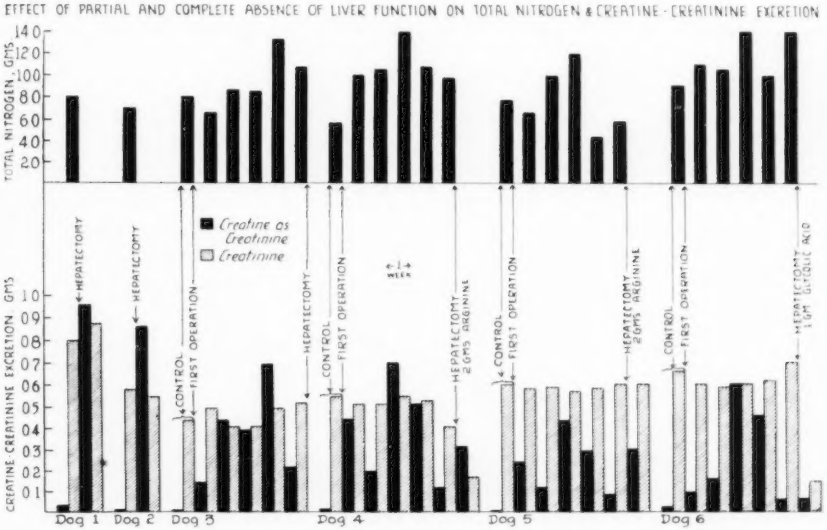

Chart 1

in physiological saline was introduced into the vein of the left hind leg and continued until just before death of the animal. At the same time a small sample of muscle tissue from the hind leg was removed for analysis of creatine. As much urine as possible was collected after the second operation, but because of the anuria in some cases and the small volume of urine in others we attach no significance to the results obtained.

Two hours after the complete removal of the liver, dogs 3 and 4 were injected parenterally with 2 grams of arginine base in 10 per cent glucose, while dog 6 received 1 gram of neutralized glycolic acid in a similar manner. Just after death a similar sample of muscle tissue was secured from the opposite hind leg of each animal and analyzed for creatine (total creatinine) by the method of Rose, Helmer and Chanutin (5).

All colorimetric determinations of creatinine in alkaline picrate were made with the Fisher electrophotometer with a maximum variation of not more than 1.5 mgm. creatinine in the 100 cc. of muscle filtrate. Filter B no. 58 was used. Using the specific creatinine enzyme of Miller and Dubos (6), we have shown on several occasions that the Jaffe reaction with alkaline picrate in muscle filtrates and urine is *specific* for creatinine.

Results and discussion. The urine results are given in chart 1. In dogs 1 and 2, which were studied just before and after the second operation (complete hepatectomy), it is seen that large increases in creatine excretion occurred. The excretion of preformed creatinine was unaffected. The excretion of preformed creatinine in dogs 3 to 6 was likewise unaffected by interference with liver function.

In dogs 3 to 6, in which ligation of the inferior vena cava and portal vein was done, the creatinuria increased with the total nitrogen until the peak was reached 3 weeks after the operation.[2] This would seem to indicate that, in the partial absence of the blood supply to the liver through the portal vein, the creatine, that is formed in other tissues, such as the muscles, heart and kidneys, does not reach the liver. Increased excretion of creatine then occurs. These results indicate that the liver is necessary to prevent creatinuria. It evidently assists in the transformation of creatine into products other than creatinine. This view is also reasonable, for we have observed in this laboratory on several occasions that the muscles are far from saturated with creatine; increases up to 138 per cent above normal have been observed. Some of these metabolic products of creatine may be glycocyamine, methyl amine and methyl guanidine, since these substances have been shown to be excreted into the urine in different metabolic conditions.

The results of the muscle creatine studies are given in table 1. Hepatectomy, *per se*, does not influence the creatine content of the muscles. On the other hand the body, in the complete absence of the liver, can convert arginine or glycolic acid into muscle creatine. We have shown in other studies (7) that arginine, or glycine with urea, furnishes the guanidine group necessary for creatine formation, while glycine, through glycolic acid also furnishes the methyl group necessary for the methylation of glycocyamine to creatine (8). Hence almost conclusive proof is furnished that the liver plays no part in creatine formation. This leaves the muscles as the chief site of its formation although there is evidence that it can also be synthesized in the kidney and heart.

In the experiments reported herewith the correlation of the creatine excretion with the excretion of total nitrogen confirms the suggestion that

[2] The urine specimens of all animals after the first operation smelled *strongly* of ammonia. However, since the determination of this urinary constituent was not carried out in the control specimens it was omitted altogether.

we have often expressed, namely, that creatine is formed as a result of protein catabolism in the body. This view has also been expressed by Terroine and his co-workers (9) and others.

Drury and his co-workers (10) and Maddock and Svedberg (11) studied the effect of liver removal in the rabbit and monkey. Uric acid, amino acids and creatine increased in the blood of the monkey, while amino acid nitrogen and creatine nitrogen increased in the blood of the rabbit. The increases in blood creatine in these cases were striking and are in agreement with the large creatinuria we have observed in our hepatectomized dogs.

In his review of the literature Mann (12) stated that amino acids, when injected into the dehepatectomized animal, are not destroyed and that the

TABLE 1

Effect of hepatectomy upon muscle creatine in the dog

DOG NUMBER	MUSCLE CREATINE AFTER HEPATECTOMY	LENGTH OF LIFE AFTER HEPATECTOMY		MUSCLE CREATINE AT DEATH	MUSCLE CREATINE INCREASE	REMARKS
	per cent	*hrs.*	*min.*	*per cent*	*per cent*	
1	0.34	16	25	0.34	None	
2	0.36	13	10	0.35	None	
3	0.26	7	22	0.26	None	
4	0.31	13	6	0.41	32.2	2 grams arginine in 10 per cent glucose injected 2 hours after operation
5	0.31	11	10	0.45	45.1	2 grams arginine in 10 per cent glucose injected 2 hours after operation
6	0.29	5	14	0.39	34.5	1 gram Na glycolate in 10 per cent glucose 2 hours after operation

amount which is not excreted into the urine can be traced to the muscles. This is additional evidence that creatine may be formed from the amino acids in this tissue.

The statement above that the liver does not change creatine into creatinine merits some brief discussion. When creatine or creatinine is injected into young rats in doses from 10 to 200 mgm., there is a retention of each of these substances which amounts to over 50 per cent of the dose injected (13). This causes a marked upset in creatine-creatinine equilibrium. Very large increases in both creatine and creatinine excretion occurred after creatinine injection, which shows that creatinine stimulates enormously this type of excretion. On the other hand, increases in muscle creatine also occurred after creatinine injection. This shows definitely that creatinine is transformed into creatine in the body.

Much to our surprise, the injection of creatine, instead of causing an increased elimination of creatinine as is generally believed, actually caused a retention of this substance. The absolute amount of creatinine retained was independent of the dose of creatine injected and varied from 74 mgm. for the 25 mgm. dose to 63 mgm. for the 200 mgm. dose. These results were confirmed on 6 workers attached to the laboratory. Thus it is evident that creatine is *not* changed into creatinine in the body.

The prevailing view that creatine formation and creatinine excretion are not influenced by the liver is confirmed in the studies reported in this paper. On the other hand, the presence of the liver is necessary to prevent the appearance of creatine in the urine. In partial or complete absence of liver function creatine formed in normal amounts accumulates in the tissues and is then excreted into the urine.

SUMMARY AND CONCLUSIONS

Interference with or destruction of normal liver functions in the dog, as produced by ligation of the inferior vena cava and portal vein or by complete hepatectomy, does not interfere with creatine formation in the muscles or creatinine excretion in the urine. On the other hand there is a large increase in creatine excretion which reaches its peak with the total nitrogen three weeks after the first operation.

Arginine and glycolic acid are transformed into muscle creatine in the complete absence of the liver.

It is concluded that the liver plays no part in creatine formation or creatinine excretion but that its presence is necessary to prevent creatinuria. This function of the liver probably assists in maintaining a uniform content of creatine in the tissues by transforming it into products other than creatinine.

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RESPIRATORY VAGAL REFLEXES AND CARBON DIOXIDE

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In order to explain the effects of drugs, such as ether which in suitable concentrations increases both the rate and depth of respiration, a more complete knowledge of the governing reflexes than was available seemed necessary. The investigation became directed into an attempt to elucidate in greater detail the working of the respiratory centre, the part played by, and the mechanism of, sensory vagal action on respiration and finally the mode of operation of drugs affecting respiration. As yet the last phase deals only with carbon dioxide.

METHODS. Rabbits alone were used, as the separate depressor nerve enabled the central vagus to be stimulated without, in the majority of cases, affecting the blood pressure. The animals were lightly anesthetised with urethane. If ether was used during the operation, at least half an hour was allowed for its dissipation. The animals were enclosed in a body plethysmograph sealed in about the neck with plaster of Paris. Respiration was recorded quantitatively with a very light gasometer recorder, and in many cases a vertical writing lever was used. The upstroke represents inspiration. Though the record is somewhat modified by the water movement, such a recorder is in our experience both more simple and more delicate than a Brodie's bellows, which readily becomes somewhat stiff.

The exposed vagus was stimulated with condenser discharges obtained from a periodically discharging gas-content triode, and amplified through an output system. The stimulus could be varied both in intensity and frequency independently. The stimulating electrodes consisted of a virtually air-tight moist chamber, with two compartments, connected electrically only by the nerve. Each compartment communicated electrically with a calomel half-cell, which connected to the stimulator leads. Such electrodes gave persistent effects with the same stimulus for hours.

Throughout the paper the words "high frequency" mean a stimulus frequency of from 50 to 75 per second; "low frequency" less than 20 per second. It was felt that the high frequency stimulation should imitate the effects produced by lung distention and the low frequency stimulation should imitate lung collapse according to the work of Adrian (1933).

In order to stimulate during the inspiratory or expiratory phases only,

a light block was strapped to the axle of the recorder, which was put into motion by light friction when the axle rotated. Each end of the block carried a light wire, the end of which was bent down at right angles and dipped into a shallow mercury-filled cup. The cups hindered the movement of the wires and block, so that the excursion of the end of each wire was only about 0.5 cm. Movement of the recording lever was in no way restricted. One cup and wire served to close the stimulating circuit during the expiratory phase only, the other during the inspiratory phase only. Either continuous (i.e., throughout the cycle), or expiratory, or inspiratory stimuli could be quickly selected by closing the appropriate contacts of a triple throw knife switch, which closed the output circuit from the stimulator.

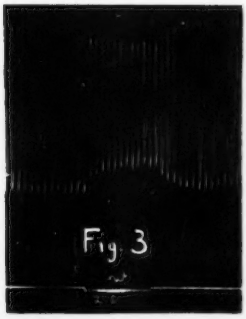
Of various methods used to increase the carbon dioxide in the inhaled air, the most satisfactory proved to be by increasing slightly the dead space of the animals' respiratory passages.

In the tracings shown, the first figure of a pair is the intensity dial setting of the stimulator; the second figure is the frequency dial setting, 0 to 4 being from 5 to 20 per second approximately; 10 represents a frequency of about 70. Intensity dial settings cannot be compared from one tracing to another because of the varying resistance and sensitivity of different nerves. On a given set-up the response is constant, and the dial settings are comparable. Small waves are mainly due to water movement in the recorder.

Simple continuous vagal stimulation. A high frequency continuous stimulus that is just suprathreshold causes a decided decrease of respiratory rate due to a prolongation of the expiratory pause, and respiration is shallower due to a less complete inspiration. There is more or less fall of expiratory tonus (fig. 1). If the intensity is increased the effects become more marked until complete cessation of respiration may occur in a state of low expiratory tonus, no efforts at inspiration being evident for some time (fig. 2a).

A low frequency continuous stimulus causes a more rapid, but shallower respiration, the decreased volume being due both to some reduction of inspiratory depth, and also to a higher expiratory tonus (fig. 3). As the intensity is increased the effect is more marked, and respiration may be arrested in a state of high expiratory tonus upon which abortive respiratory movements may be evident (fig. 2b). It is impossible however, to increase the rate beyond a certain degree, in some cases about 25 per cent. Further increase in intensity continues to reduce the volume and increase the expiratory tonus, but does not increase the rate further (table 1).

Ventilation is reduced with both types of stimuli. For low frequency effects, a greater intensity of stimulation is required than for high frequency effects.



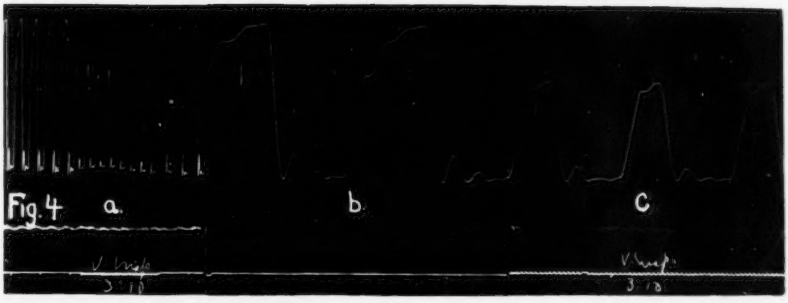


Fig. 1. Effect of vagal stimulation with moderate intensity high frequency (70 per sec.) continuous stimulus; rate decreased; inspiration reduced; tonus lowered. Note increasing depth with CO_2 accumulation.

Fig. 2a. Effect of strong intensity high frequency continuous stimulus. Respiratory inhibition in state of low expiratory tonus.

Fig. 2b. Strong intensity low frequency continuous stimulus. Respiratory arrest in state of high tonus with abortive respiratory movements at increased rate over normal.

Fig. 3. Effect of moderate intensity low frequency (10 per sec.) continuous stimulus. Rate increased 11 per cent. Expiratory tonus raised. Inspiration somewhat reduced.

Fig. 4a. Effect of inspiratory high frequency stimulus of moderate intensity. Rate increased 45 per cent. Inspiration markedly reduced. Tonus practically unchanged.

Fig. 4b. Normal tracing with rapid drum.

Fig. 4c. Same stimulus as in a, with fast drum, showing the rate increase mainly due to shortened inspiratory phase, but slightly due to shortened expiratory pause.

Fig. 5a. Effect of inspiratory low frequency stimulus of moderate intensity. Rate increased 12 per cent. Inspiration somewhat reduced. Tonus raised.

Fig. 5b. Normal tracing, rapid drum.

Fig. 5c. Same stimulus as in a, with rapid drum, showing that the rate increase is partly due to shortened expiratory phase, partly to shortened inspiratory phase.

Stimuli applied during inspiration only. A high frequency inspiratory stimulus reduces each inspiration making the respiration shallower. At the same time the duration of the inspiration is markedly shortened and also the duration of the subsequent expiratory phase is somewhat shortened so that there is a definite increase of rate. This increase in rate is mainly due to the reduction of the duration of each inspiratory period. The expiratory tonus level is unchanged as a rule with high frequencies, occasionally raised, especially if the intensity is high (fig. 4).

Inspiratory stimuli of low frequency produce similar effects, except that the expiratory tonus level is invariably raised (fig. 5). A stronger intensity is necessary to produce a given effect with low frequency inspiratory stimuli than with high frequencies, and a greater increase of rate is attainable by increasing the intensity of a high frequency inspiratory stimulus than with a low frequency.

Stimuli applied during expiration only. High frequency expiratory stimuli cause a prolongation of the expiratory pause, thus decreasing the

TABLE 1

Experiment June 20. Effect of progressive increase in intensity of low frequency continuous stimuli

STIMULUS (DIAL SETTINGS) INTENSITY: FREQUENCY	RATE	VOLUME	VENTILATION
	per cent	*per cent*	*per cent*
2 :1	+10.6	−31	−23
2.4:1	+12.5	−34	−26
3 :1	+20	−40	−29
4 :1	+13.5	−45	−37

rate. Expiratory tonus falls more or less. When an inspiration does break through the inhibition (CO_2 accumulation) it is of nearly normal depth (fig. 6). Occasionally the depth is slightly reduced. Increasing the intensity of the stimulus leads to a greater prolongation of the expiratory pause and therefore greater slowing of respiration. The ventilation is markedly reduced.

Low frequency expiratory stimuli hasten the onset of the next inspiration and shorten the expiratory pause, thus increasing the respiratory rate. Expiratory tonus rises. Each inspiration is of less volume than formerly, due in part to the higher expiratory tonus and in part to a slightly shallower inspiration (fig. 7). As the intensity is increased the effects become more marked. The ventilation is invariably reduced because the reduction of inspiratory volume is proportionally greater than the increase of rate. Again, the threshold of stimulus intensity is lower for high frequencies than for low.

In general high frequency stimuli cause a prompt onset of effect when

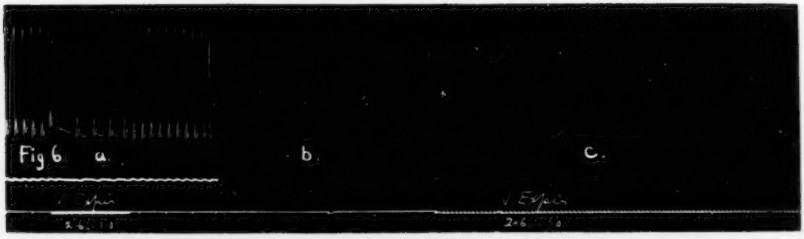
they are applied, and a prompt cessation of effect when they are stopped. With low frequencies there is an increasing effect over a period of a few respirations when the stimuli are applied and also some persistence of effect for the same time after cessation of the stimuli.

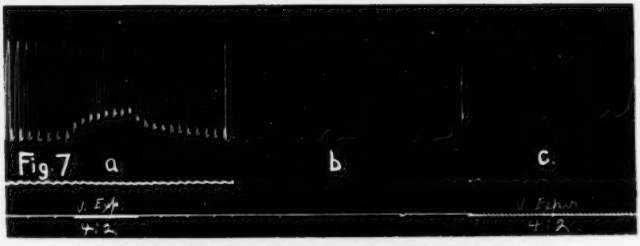
Effect of simultaneous stimulation with low and high frequencies. It was felt that the increase of rate that occurs with both high frequency inspiratory stimuli and low frequency continuous stimuli was due to two different mechanisms, because of the different effects on tonus, the different thresholds of intensity and of other less marked effects. To test this the two types of stimuli were first applied separately through separate electrodes fixed to the same vagus nerve, and then simultaneously. It was found that the increase of rate that occurred when both a low frequency continuous stimulus and a high frequency inspiratory stimulus were applied simultaneously, was almost exactly the sum of the increases that occurred when the two types of stimuli were applied separately. The combined effect upon expiratory tonus was intermediate between the individual effects. It seems reasonable to believe, therefore, that two entirely different mechanisms are involved in stimuli producing low and high frequency effects, each of whose individual actions are independent of the other. Whether these two mechanisms are due to some selective central action, or whether they are the result of two different sets of fibres in the vagi being affected, cannot be determined. The latter seems more probable.

Action of carbon dioxide. It is well known that an increase in CO_2 in the inspired air causes an increase in the depth of respiration both when the vagi are intact and when severed. Tracings made with a fast drum in the course of these experiments show further that the slope of the inspiratory curve is invariably steeper during CO_2-air inhalation than without CO_2.

An increased CO_2 in the inspired air causes a decrease in the effectiveness of the vagal reflexes as previously noted by Gesell and Moyer (1935b). With any type of stimulation, each inspiration is reduced less during CO_2 inhalation than without CO_2, low frequency stimuli increase the rate less during CO_2 inhalation than without CO_2 and all types of stimuli reduce the ventilation less during CO_2 inhalation than without CO_2. But when the stimulus is applied during inspiration only, especially when of high frequency, its effect upon the ventilation is remarkably changed. Normally such a stimulus reduces the ventilation considerably in spite of the increase in rate. But during CO_2 inhalation it causes an increase in the ventilation (fig. 8). When the CO_2 in the inhaled air is increased to about 5 per cent, inspiratory high frequency stimuli are no longer very effective in reducing the volume of each inspiration, though they still cause an increase in respiratory rate, so that the ventilation increases. The importance of this in the intact body will be discussed later.

Vagal section and respiration. It is of course well known that upon





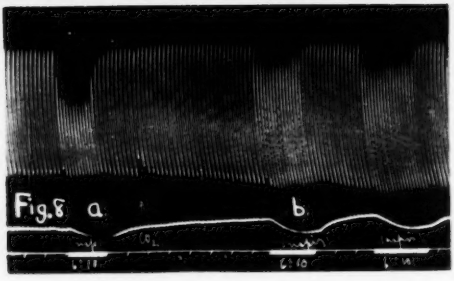


Fig. 6a. Effect of expiratory stimulus of moderate intensity and high frequency. Rate markedly decreased. Inspiration of approximately normal depth. Tonus practically unchanged.

Fig. 6b. Normal tracing, fast drum.

Fig. 6c. Same stimulus as in a, with fast drum, showing decreased rate entirely due to prolongation of expiratory phase.

Fig. 7a. Effect of expiratory low frequency stimulus of moderate intensity. Rate increased 17 per cent. Tonus raised. Very slight reduction of inspiration.

Fig. 7b. Normal tracing, fast drum.

Fig. 7c. Same stimulus as in a, with fast drum, showing increase in rate entirely due to shortening of expiratory phase. Inspiratory phase unchanged.

Fig. 8. Effect of carbon dioxide.

Fig. 8a. Inspiratory stimulus, moderate intensity, high frequency. Rate increased 40 per cent. Inspiration markedly reduced. Ventilation reduced 24 per cent from normal.

Fig. 8b. Same stimulus during CO_2-air inhalation. Rate increased 30 per cent. Inspiration only slightly reduced. Ventilation increased 21 per cent. The fall in blood pressure is unusual.

section of either or both vagus nerves, the respiration is slower and deeper. An analysis of a large number of tracings in which one or other vagus was quickly and cleanly severed or cocainized when CO_2 was not increased, showed that in spite of the slower rate the ventilation increased for a period of a few minutes (table 2). It subsequently falls off to approximately its normal value, although each respiration is still of greater volume than the normal. During the period of increased ventilation, considerable CO_2 must be blown off, and since each breath remains deeper than normal, a lower CO_2 level must be established and maintained in the blood stream. In other words, a normal ventilation is maintained after cutting of the vagus nerves at a lower CO_2 level than before. It would appear therefore that in the normal state the impulses travelling up the vagi on the whole

TABLE 2

Effect of cutting vagi nerves under low and increased alveolar CO_2 concentrations

	DATE	VENTILATION BEFORE VAGAL SECTION		VENTILATION 30 SECONDS AFTER VAGAL SECTION
		cc.		cc.
Alveolar CO_2 low	May 27	887	Cut left vagus Right vagus intact	988
	May 28	624	Cut right vagus Left vagus already cut	694
Alveolar CO_2 increased	June 29	1,100	Cut left vagus Right vagus intact	1,040
	July 18	1,288	Cut right vagus Left vagus already cut	1,000

somewhat reduce the irritability of the respiratory centre to carbon dioxide.

Such occurs if the CO_2 is within normal limits (taking the character of the respiratory movements as a criterion). But if the CO_2 is increased before the vagus is cut, the cutting results in a reduced ventilation, though the CO_2 is maintained the same (table 2). This is in agreement with the observations with high frequency inspiratory stimuli, which decrease the ventilation normally, but increase it if the CO_2 is high. Further corroboration of the change of the vagal reflexes from an inhibiting influence when the CO_2 is low, to an augmenting influence when the CO_2 is high, is seen by the following. A normal rabbit's respiration was 650 cc. Under CO_2 (increased dead space; both vagi intact), the ventilation increased to 1162 in 5 minutes. The CO_2 was left off for a short time and the ventilation fell to 730 cc. The left vagus was cut and the ventilation increased to 768 cc.

for a short time and later fell to 676 cc. The same CO_2 (one vagus now cut) only caused the ventilation to increase to 876 cc. in 5 minutes. The augmenting influence of one vagus had been removed.

The explanation lies in the fact that under the action of increased concentrations of CO_2 the vagal stretch reflexes do not reduce each inspiration much, whereas their effectiveness in increasing the rate is maintained. Normally, although they bring about a faster rate, they are even more effective in reducing the volume of each breath.

Although the results described were obtained with remarkable constancy, it was observed that an occasional vagus nerve failed to produce the usual effects either upon cutting or upon stimulation. In one case, cutting both vagi failed to alter the respiration in any way. Usually, when one vagus failed to produce the expected effects, the other did produce them. Further, on one or two occasions it was found that typical low frequency effects could be obtained with frequencies up to 50 per second, and that much higher frequencies than usual were required to obtain high frequency effects, in one case about 200 per second. Obviously the limits of frequency of the stimuli used to obtain typical low and high frequency effects cannot be sharply defined, as the above variability indicates. The frequencies chosen (cf. methods), have been found to give typical effects in all but a few cases. It is highly probable that frequencies intermediate between those used would give a variety of effects, depending upon the particular nerve and animal being stimulated. Therein may lie the explanation for the variability of respiratory effects in response to vagal stimulation reported in the literature.

In all about 50 rabbits have been used.

Discussion. There seems to be an accumulating amount of evidence to show that the cells of the respiratory centre are automatic, the automaticity depending upon the CO_2 present. For descriptive purposes it seems reasonable to consider the rate of respiration as depending upon the rate at which the cells become charged by the action of CO_2 to the point where they automatically discharge. Contrary to most of the results obtained by Scott (1908) it has been observed in these experiments that even with one or both vagi cut, there is an increased respiratory rate with increased CO_2, though it is less when the vagi are severed than when intact. Also it seems apparent that the depth of inspiration will depend, not upon the rate of charging of the respiratory cells, but upon the number of them that discharge more or less synchronously throughout the inspiratory phase. After any discharge, a period must elapse before the cells are recharged and another inspiration begins. On this basis the greater inspiratory depth that occurs with increased CO_2, together with the fact that the curve of inspiration is steeper, is evidence that a larger number of respiratory cells than normally is being brought into action. Where gasp-

ing respiration involving the accessory muscles occurs, it is evident that more cells are discharging. The stretch reflex seems to cut short the discharge from all the cells taking part in the inspiratory act, and increases the rate mainly by shortening each inspiratory phase. It appears as if such afferent impulses suddenly make the respiratory cells no longer sensitive to the concentration of CO_2 bathing them, and they cease to discharge. At the same time the stretch reflexes shorten somewhat the subsequent expiratory pause, as though the respiratory cells, after being made less sensitive to the CO_2 surrounding them for a short time, pass through a period when they are more sensitive, and so discharge sooner. Adrian (1933) explains the shortening of the expiratory pause upon the basis that the cutting short of the inspiratory discharge leaves some "active material" available, which is applied toward the subsequent inspiration and so makes the cells reach the discharging level sooner. This does not seem to be an adequate explanation for two reasons. First, when a vagus is cut there is an immediate change in the ventilation, which may be an increase or decrease, depending upon whether the CO_2 being inhaled is low or high. One would expect no change in the ventilation. Secondly, according to such an explanation one would expect the cutting short of the inspiratory phase to be exactly equal to the shortening in duration of the expiratory phase. The increase in rate due to the stretch reflexes should be due to an equal reduction in the durations of the inspiratory and expiratory periods. It was shown that this is not so, and that the increased rate is largely due to a shortening of each inspiratory period, and slightly to a shortening of each expiratory period. It is impossible yet to formulate any justifiable explanation for this action of the stretch reflexes.

High frequency stimuli applied in expiration or continuously (equivalent to stretching the lungs as in Adrian's (1933) experiments) delay the onset of the next inspiration. This appears to be merely an exaggeration of the cutting short of inspiration when the stimuli are applied in the inspiratory phase only, in that they prevent the cells from discharging until CO_2 accumulates further. Again it appears as if the high frequency stimuli make the respiratory cells no longer sensitive to the concentration of CO_2 bathing them, and they fail to discharge until more CO_2 accumulates.

Low frequency stimuli lead to an increase in respiratory rate, as though the cells discharged more rapidly to the CO_2 present. But the slope of the inspiratory curve is less steep, as though the cells were discharging less synchronously. In other words, some of the cells have been made more sensitive to CO_2, and discharge in response to a lower concentration than required normally, and so set up earlier inspirations. It appears, therefore, that in response to appropriate stimulation, certain fibres in the vagi

can arouse part of the respiratory centre to inspiratory activity, independent of the normal rhythm. Possibly such fibres correspond to the so-called sensory collapse fibres of the lungs. The results offer evidence of the presence of vagal accelerator fibres in the sense discussed by Hammouda (1935) and others.

The rise in base line that occurs with all low frequency stimuli may be the result of the direct action of the vagal impulses upon the respiratory centre. On the other hand it may be due to some more indirect connections. One must recollect that the vagus impulses pass to the nucleus of the tractus solitarius, and thence to the respiratory cells; and they may possibly pass from the nucleus to the anterior horn cells supplying the respiratory muscles, and thus affect their tonus. It might also be that the asynchronous discharge apparent with all low frequency stimuli, and giving rise to the earlier slow inspiratory movements, might occur at the anterior horn cells rather than at the respiratory centre.

It has been noted frequently that vagal stimulation may at times decrease the respiratory rate and at times increase it. The work of Rosenthal, cited by Gesell and Moyer (1935a), demonstrated a slowing of respiration when stronger intensities of stimuli were used, and attributed such effects to a spread of stimulus to the superior laryngeal nerve. Others have since obtained slowing with weak stimuli. Hammouda (1935) showed that in one of his experiments the respiration was accelerated with one stimulus and slowed with another of higher frequency, and suggested that such results might have been due in part to nerve changes. He could not duplicate this. It now seems probable that the variability of results previously reported is due to the fact that the conditions of the experiments, especially with regard to the frequency and intensity of the stimuli, had not been adequately controlled. In fact, contrary to Rosenthal's opinion, it has been shown in these experiments that a lower intensity is necessary to decrease the respiratory rate with the appropriate frequency, than to increase it with the proper frequency.

It seems unlikely that the carotid sinus mechanisms would play any part in these experiments. In those cases where CO_2 was not used nothing was done to arouse carotid sinus reflexes, and in those cases where CO_2 was increased, only low concentrations were used, and it seems certain that under such conditions its effects are central.

It has long been known that the increase of ventilation in response to a given increase of CO_2 is greater if the vagi are intact than if they are severed, because there is a great increase in rate as well as depth in the former case, but less increase of rate in the latter case. Further, as was shown above, cutting the vagi when the CO_2 was high led to a decrease of ventilation, whereas cutting when the CO_2 was low led to an increase. Similarly, inspiratory stimuli were shown to increase the ventilation when

the CO_2 was high, but to decrease it when the CO_2 was low. It appears that the respiratory centre is rather restrained in its activity by the stretch reflexes when the CO_2 is low, and augmented by the same reflexes when the CO_2 is high. Such results throw considerable light upon the important part played by these reflexes in their dual rôle as a conservation mechanism when the body is quiet, and as a promptly acting emergency mechanism increasing the ventilation, when the CO_2 is raised even slightly. The reflexes amplify the ventilation in greater proportion than the increase in CO_2 alone would do, and so increase the flexibility and promptness of action of the respiratory movements. One wonders how much the loss of reflex control in cases where lung movement is restricted, or where there has been tissue damage, such as in pneumonia and emphysema, is responsible for the laboured breathing, and the necessity for voluntary control of the respiration and its attendant distress. Finally it is worth while to point out that the vagal reflexes, influencing the respiration as they do, serve as physiological buffers in maintaining a more constant blood CO_2 and pH than would otherwise be possible. When the CO_2 is low, and the pH tending to be high, the respiration, especially the depth, is restrained by vagal reflexes and the accumulation of CO_2 thereby aided. But as the accumulation proceeds, the ventilation becomes less and less restrained by the same reflexes, and indeed becomes augmented by them when the CO_2 is high enough, so that the excess CO_2 is blown off and a pH swing in the other direction avoided.

SUMMARY

The respiratory effects of vagal stimulation were studied and it was found that the frequency of the applied stimulus determines to a large extent the result. The manner in which the stretch reflexes of the lungs operate, both in quiet respiration and under the action of increased concentrations of CO_2 was observed, and corroborated by the behaviour of the vagal reflexes when subject to various types of stimuli. Finally the possible modes of action of the effects observed, and their practical importance, are discussed.

I wish to take this opportunity to thank Prof. V. E. Henderson for suggesting this problem and for his helpful criticism and constant advice during this work.

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ISOLATION AND ANALYSIS OF EXTRACELLULAR MUSCLE FLUID FROM THE FROG

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Many calculations of various types, pertaining to the volume of the extracellular fluid in muscle, have been reported. However, there are found in the literature no reports of the actual isolation of samples of such fluid from muscles, though studies have been made on lymph fluid, spinal fluid, ascites and peritoneal fluid from a variety of animals and man.

At the suggestion of Dr. Emil Bozler, attempts were made to remove the extracellular fluid from muscles by centrifugalizing at high speeds. The muscle was attached by means of a stout thread to a stopper in a centrifuge tube. The tube itself was filled with liquid petrolatum in which the muscle was suspended. The whole arrangement was then placed in the centrifuge and whirled at high speed. Any fluid, separating from the muscle, collected at the point of the tube and was measured by means of graduations on the tube. Fluid could be separated from the muscle by this method, but analysis of the samples showed that there was almost as much potassium present in the fluid so collected as there is in muscle cells. This proved quite definitely that samples so obtained were not purely extracellular in origin but contained to a large extent the constituents of muscle cells.

In a paper by Fenn and Maurer (1935) this author made a brief preliminary report of a method which he had devised in this laboratory. At the time of the report it was not definitely proved that the samples were truly extracellular in origin, but this has since been done. The following report contains an account of the method used in obtaining the samples, and the analyses which have been made on the fluid itself.

METHOD OF EXTRACTION OF FLUID. It was found in 1934 that a clear, straw-colored fluid could be collected when tiny capillary tubes were inserted into the muscles of the frog.

Throughout this entire work the main tool employed was thin-walled, Pyrex, capillary tubing of very small inside diameter. The tubing used was machine drawn by the International Resistance Company of Philadelphia, Pennsylvania. Two sizes were employed, as follows: the smaller

measured, outside diameter 0.5 mm. and inside diameter 0.3 to 0.35 mm., the larger measured, outside diameter 0.8 mm., and inside diameter 0.6 mm. The smaller size was used exclusively in collecting the fluid and the larger was used in the analyses which followed.

The procedure employed in extracting the fluid was as follows: Using a pithed frog, the skin over the upperleg muscles or over the gastrocnemius muscle was incised and reflected so as to leave the surface of the muscles entirely exposed. With a piece of filter paper or dry, neutral gauze, the lymph was wiped from the surface of the exposed muscles so as to leave them fairly dry. At this point, a piece of the smaller tubing (0.3 mm.) about 3 inches in length was inserted lengthwise into the muscle at the knee end in the case of the sartorius or quadriceps, or at the ankle end of the gastrocnemius. The muscle was held fairly firmly between thumb and index finger, and the capillary was pushed the entire length of the muscle without puncturing the opposite end. It was then withdrawn slowly, while the fingers still held the muscle firmly between them. The muscle was not squeezed at any time during the procedure but only very gentle pressure was applied. The muscle remained in situ in the frog's leg and the circulation remained intact throughout the experiment.

Upon withdrawal of the tube, there was generally found within it a small amount of clear, straw-colored fluid. The greatest quantity collected amounted to 1.8 cu. mm., while many samples were as small as 0.5 cu. mm., the general average being about 1.0 cu. mm. Regarding the removal of this fluid, there are certain factors which must be taken into account. First, it was found that the fluid is much more easily obtained from winter frogs than from summer frogs. It is possible to obtain small amounts from summer frogs, but in general not enough for satisfactory analysis. It is generally regarded that the fat stores are low in winter frogs and that the water content of the body musculature is higher than in summer. Whether or not this is produced by a general condition of hunger is not known. Also, one cannot use with any degree of success a frog which is afflicted with so-called "red leg." In such frogs there are numerous small petechiae and hemangiomae which have formed on and about the very small blood vessels lying between the muscle cells. On inserting the capillary into the muscle it is almost impossible to avoid breaking into these formations and drawing blood from them. This can be noted readily enough by the color of the fluid obtained. Samples containing blood have in all cases been discarded. The capillaries have all been accurately calibrated before being used so that, by measuring the length of the column of fluid obtained, one could easily calculate the volume of the sample. The measurements were made on an accurately constructed steel rule, on which the length could be estimated fairly easily to 0.1 mm. The error in reading was probably of the order of 2.0 to 3.0 per cent.

Having obtained such samples satisfactorily, it now remained to determine whether it was extracellular in origin, and if so, what some of its characteristics were.

ANALYSIS OF FLUID. In order to determine whether fluid so obtained was extracellular, several methods of analysis were employed.

Histological analysis. The scheme in this type of analysis was to insert into the capillary tube, previous to inserting it into the muscle, a fine human hair. The hair was allowed to remain in the tube while it was being inserted, and in this case the capillary was made to pierce the opposite end of the muscle where the end of the hair protruding from the tube was caught with forceps and held steadily while the tube was withdrawn. This allowed the hair to remain and thus mark the path that had been taken by the tube through the whole length of the muscle. The hair was then cut off at either end close to the surface of the tissue; the muscle was placed in fixative, and later mounted and sectioned. The sections were

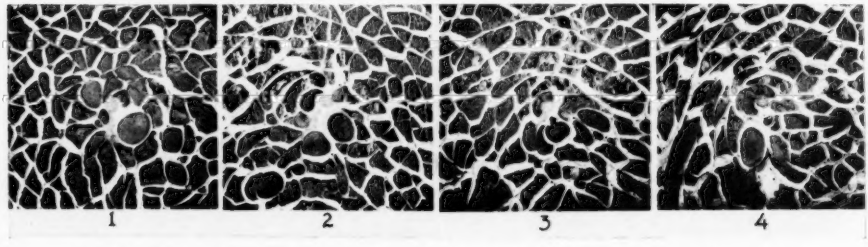

Fig. 1. Path of capillary tube through muscle defined by cross section of hair. Hair section is small, light-colored, oval-shaped body in center of each muscle section.

not taken in complete serials but only 6 or 8 serially cut sections were taken at 15 to 20 different points along the whole length of the muscle. These sections were mounted and were then examined to locate the hair. By the position of the hair in relation to the muscle fibres, it was hoped to reveal whether or not the tube had penetrated or torn any of the cells along its path, and in this way determine qualitatively, at least, whether or not the samples obtained were purely extracellular in origin or whether they contained constituents from torn cells.

A typical picture of the hair in relation to the cells surrounding it, is shown in figure 1.

Examination of 150 such sections from a number of muscles showed the following: 1, the hair in no case was found to have penetrated any muscle cells; 2, even though the substance of the muscle fibres had shrunk away from the sarcolemma while being fixed and sectioned, the hair was not seen to be lying inside the sarcolemma (between it and the shrunken cell

substance); 3, even in cells which had not shrunk away from the sarcolemma and which lay close to the hair, the cell boundaries were still intact and the cell nuclei could be seen lying undisturbed about the periphery; 4, in cases where the tube had come up against a cell obliquely, the cell was never seen to be torn, but instead, as is demonstrated very clearly in figure 1, it was pushed aside, the tube indenting its surface and changing its shape. In figure 1, reading from 1 to 4, one of the cells is made to change shape in this way. The fifth section (not shown) showed the hair again as it appears in section 1, with the cell in question back in its original form.

These findings were taken as fairly clear evidence that the fluid obtained in this manner was extracellular and did not contain any cell material. A preliminary report of these hair-section findings appeared in the article by Fenn and Maurer (1935). That it is possible to insert the tube without tearing or puncturing any of the muscle fibres has been confirmed in a personal communication with Dr. F. J. M. Sichel (1936), who stated that in trying to make microinjections into single cells, the sarcolemma is exceedingly tough, requiring a special technique to puncture it with a microneedle. He agrees with these findings unquestionably. To make the story even more convincing, the capillaries were inserted without first fire-polishing the ends. This made no difference, for the tissue sections still showed the same picture that they had shown when the tubes were fire-polished.

Chloride analysis. This method of analysis required the use of accurate micro-determinations for chloride. Two possible methods were considered; the drop titration of Wigglesworth (1937), and the electrometric titration of Eggleton, Eggleton and Hamilton (1937). The second was chosen because of its greater simplicity. This method is essentially a concentration cell set up as follows:

$$\mathrm{Ag} \;\Big|\; \begin{matrix}\text{"Unknown" Cl solution in} \\ \text{N/4 } CCl_3COOH\end{matrix} \;\Big|\; \begin{matrix}\text{N/4 } CCl_3COOH \text{ saturated} \\ \text{with AgCl}\end{matrix} \;\Big|\; \mathrm{Ag}$$

With a galvanometer in the output from the cell, the deflection due to the presence of chloride ions is registered. By titrating the fluid in the "unknown" half-cell with $AgNO_3$ the chloride ions are bound and the galvanometer deflection becomes less until at the point where all the chloride is combined as AgCl, the galvanometer registers zero. Further addition of $AgNO_3$ causes a deflection in the opposite direction, denoting the presence of Ag ions.

This method was modified by the author and it was found that it could be made quite accurate for determinations of chloride in blank solutions as small as the amounts of extracellular fluid obtained. The system was kept essentially in its original form. The electrodes were reduced greatly in size, and special small titrating vessels and a special burette were con-

structed. The output from the cell was amplified in order to magnify the galvanometer deflection.

The electrodes were made of fine silver wire, while the salt bridge between the "unknown" and reference half-cells was drawn out to a capillary point. A direct-coupled amplifier, designed by Dr. A. C. Young of this laboratory, was used.

The titrating vessels were made by cutting the bottom end out of an ordinary bacteriological agglutination tube, so that there remained a vessel about $\frac{1}{4}$ inch deep. Five of these were made and mounted in a block of plasticine. One was used to contain fluid from the bridge with which to balance the system, and the other four were used for unknown samples. The burette consisted of a 1 cc. Tuberculin syringe mounted on a screw manipulator built of heavy brass. The thread of the screw was very fine, so that one full turn of the milled head caused the delivery of approximately 0.008 cc. The head was machine-divided into eight sections, each one delivering 0.001 cc. The column leading from the syringe was made of the larger (0.6 mm.) capillary tubing described, the tube being sealed into the syringe opening. The whole tube was approximately 16 inches long, 2 inches being turned down at the free end, and the point being drawn out to a very fine opening. The syringe was half filled with mercury, which was expelled into half the length of the capillary burette. The end of the burette was dipped into 0.01 N $AgNO_3$ (carefully preserved in black bottles and frequently standardized against standard NaCl solution) and the mercury drawn back into the syringe until the $AgNO_3$ was drawn up to the midpoint of the burette, care being taken that the solution should never enter that part of the tube which had been in contact with the mercury.

The capillary salt bridge, the silver electrode for the "unknown" half-cell, the free end of the burette and a capillary tube connected to a compressed air line through a trap bottle were all mounted on a set of clamps attached to an elevator stand, so that all these units could be raised and lowered simultaneously from or into the titrating vessels. The open end of the burette extended into the solution to be titrated, thus allowing accurate delivery of extremely minute quantities. The capillary tube attached to the air line was used to stir the fluid in the titrating chamber. The stream of air was played from an angle onto the center of the surface of the fluid, causing a double rotation of the liquid much like the effect of an egg beater. This stirring was sufficient to keep all precipitate formed during the course of the titration in suspension and in constant motion.

It was found that any amount of trichloracetic acid added to the "unknown" half-cell caused no variation in the galvanometer reading. However, it was also found that the addition of any amount of water to the half-cell caused a displacement of the reading away from the chloride deflection

(toward the silver side). Therefore any solution, such as the $AgNO_3$ (0.01 N used for the titration) made up in water, and added to the chloride-containing solution in the "unknown" half-cell, caused a movement of the galvanometer needle toward the silver side. In the case of $AgNO_3$ there was a movement due to the combination of Ag and Cl ions to form AgCl and at the same time there was an additional deflection in the same direction, due entirely to the water added. It was therefore necessary to calibrate the system for dilution by water. This was done simply by adding known amounts of double, glass-distilled water and recording the displacement of the zero point. This done, the end point of a titration was not the real zero of the galvanometer but a new zero, which was determined by the amount of water dilution brought about in performing the titration. Since the addition of trichloracetic acid caused no displacement, dissolving the $AgNO_3$ in a N/4 solution of the acid was tried. There is, however, free chloride, as impurity, present in the acid. It is almost impossible to titrate this Cl, because the acid itself makes the reading of a color end point very inaccurate. This free chloride combines with the $AgNO_3$ of the standard, precipitating it as AgCl, thus changing the actual value of the standard by an unknown amount.

This dilution calibration being completed, a series of blank titrations was run to determine the probable error of the system. With no protein present in the blank standard solutions, the probable error was equal to $\pm$ 4.8 per cent for a single determination. Blanks made up from dog serum and diluted so that the protein content was not over 2 per cent, were accurately titrated by the method of Manery, Hastings and Danielson (1938), this value being taken as standard. Titrations of such solutions by the micromethod gave a probable error of $\pm$ 4.7 per cent for a single determination. It is apparent from these calculations that the presence of protein up to 2 per cent had no effect on the accuracy of the method.

It was desired to determine the chloride concentrations in extracellular fluid samples and compare them with the chloride concentrations in the serum of the same animals. From these it was hoped that it might be shown that the ratio, serum chloride to extracellular fluid chloride, would agree with the ratios shown to exist by Hamilton (1925), Henderson (1921), Loeb, Atchley and Palmer (1921–22), Henderson, Bock, Field and Stodard (1924), Van Slyke (1926) and others, in such as spinal, ascites, lymph and peritoneal fluids.

Accordingly, extracellular fluid samples and blood samples were collected from 25 frogs. The chloride in the extracellular samples was titrated as described, first diluting the fluid about 5 times with N/4 trichloracetic acid (CCl_3COOH) in order to precipitate the proteins present. Without this preliminary precipitation, the proteins made a gummy mass in the bottom of the titrating flask making it difficult to stir the solution suffi-

ciently. The titration is not interfered with, however, by the presence of proteins in concentrations as high as appear in frog blood serum. The blood samples were centrifugalized and allowed to clot, the serum being withdrawn in an accurately calibrated 1 cc. Tuberculin syringe. After

TABLE 1

Ratios of serum chloride to extracellular fluid chloride and chloride spaces of frog muscle

Cl IN SERUM	Cl IN EXTRACELLULAR FLUID	Cl IN MUSCLE	$\frac{\text{Cl IN SERUM}}{\text{Cl IN EXTRACELLULAR FLUID}}$	Cl SPACE*
mgm./100 cc.	*mgm./100 cc.*	*mgm./100 gm.*		*per cent*
279	284	49	0.982	17.2
322	302	39	1.066	12.9
295	333	57	0.886	17.1
289	304	26	0.951	8.5
260	257	33	1.012	13.0
306	302	49	1.013	16.3
279	264	51	1.057	19.4
307	299	63	1.027	21.2
276	278	53	0.993	18.9
296	317	64	0.934	20.2
270	284	54	0.951	19.1
297	268	52	1.108	19.4
269	302	61	0.891	20.2
284	313	72	0.907	22.9
291	280	73	1.039	26.0
308	261	64	1.180	24.4
225	256	77	0.879	30.2
279	302	60	0.924	20.0
298	278	63	1.072	22.7
282	285	63	0.989	22.0
279	271	54	1.030	19.8
266	283	56	0.940	19.9
303	302	61	1.003	20.1
285	288		0.993	19.6

$$\text{Average of } \frac{\text{Cl in serum}}{\text{Cl in extracellular fluid}} = 0.99 \pm 0.01\dagger$$

* Per cent Cl space $= \frac{\text{Cl in muscle}}{\text{Cl in extracellular fluid}} \times 100$; assuming all chloride to be extracellular.

† ± 0.01 is the probable error of the mean.

measurement, the samples were discharged into 50 cc. centrifuge tubes for chloride analysis by the method of Manery et al. (1938). Samples of the muscles, from which the extracellular fluid samples had been drawn, were also taken and analysed for chloride by the same method.

The results of these chloride titrations and the calculations made from them are shown in table 1.

It is seen in table 1 that the ratios, serum chloride to extracellular fluid chloride, show an average value of 0.993 ± 0.010, which agrees very well with such values from other fluids as reported in the monograph by Van Slyke (p. 33, 1926). It would appear, then, that the samples obtained by this capillary method are ultrafiltrates of blood plasma and that the chloride ratios agree with those already described for other fluids. This agreement, and the fact that the chloride ratios practically equal 1.0, constitutes additional proof for the extracellularity of the fluid collected.

This table also shows that the interspace volume obtained from the ratios of muscle chloride to extracellular fluid chloride give an average value of 19.6 per cent (of the weight of the muscle) with a mean probable error of ± 0.4 per cent. This value also corresponds with similar values reported by other investigators.

Erythrocyte counts. To obtain still further proof, counts of the erythrocytes in the samples were made before chloride analysis. The method was to allow the tube containing the sample to lie flat on the table for a short time (the tubes being sealed, no evaporation could take place). After a short time the tubes were laid on a glass slide, bottom side up. In this manner the cells which had settled to one side were plainly visible and could be counted before they settled again to the other side. Counts were made on the whole samples and the number of cells per cubic millimeter in each was calculated.

Twelve such counts, showing extreme values of 16 and 785 and an average of 265 red blood cells per cu. mm. of fluid, compared with counts from frog blood (500,000 to 1,000,000 cells per cu. mm.), demonstrate clearly that this fluid was not collected from torn or punctured capillaries in the muscle, but is a sample of the fluid already existing between the muscle cells.

These 3 types of analyses were considered sufficient to establish the fact that the samples collected by this method were truly extracellular, and contained no material from the muscle cells.

pH measurements of extracellular fluid. Measurements on the pH of this fluid have also been made. These were reported in *Protoplasma* **24:** 1935, in the article by Fenn and Maurer. These measurements were obtained by the use of capillary colorimetry. The results of these determinations show that the fluid surrounding normal uninjured muscle cells is as alkaline as pH 7.4, corresponding with the pH of blood and being higher by about 0.5 pH unit than the most reliable figures for the intracellular pH of muscle (pH 6.6–6.8, Schmidtmann, 1924 and 1925, and Chambers, 1932; pH 6.9, Fenn and Maurer, 1935).

pH values for fluid from injured and iodoacetate-treated muscles were also reported (same article, page 341).

Determination of the protein in extracellular fluid. That protein is present in this fluid in appreciable quantities was shown during the chloride titrations, when trichloracetic acid was added to the samples. Estimation of the amount of this protein was done gravimetrically as follows.

Lengths of the smaller tubing (0.3 mm.) were accurately weighed on an analytical balance. Fluid samples were collected in them in the usual manner and the tubes were weighed again, the weight of the fluid being obtained by difference. Longer lengths of the larger capillary tubing (0.6 mm.) were weighed on a microbalance to $\pm$ 0.002 mgm. Handling them with specially cleaned forceps, the tubes were partially filled, by capillary attraction, with N/4 trichloracetic acid. The column of acid was then drawn up into the center of the tube by inverting it, allowing the acid to flow by its own weight. One end of the tube was sealed off in a quick, hot flame. This did not change the weight of the tube, as was shown by weight checks before and after the closure. The acid was then spun down into the point of the tube, by centrifugalization in a buckskin lined centrifuge tube, buckskin being used to prevent the accumulation of lint on the capillary. At this time a shoulder of DeKhotinsky's cement was made on the capillary containing the sample, at such a point along its length that the inserted end of the sample tube was held about 2 mm. above the meniscus of the trichloracetic acid in the larger tube. The combined tubes were then spun for a very short time. The small tube was removed, filled by capillary attraction with acid, reinserted and spun again for a short time. The sample tube was again removed and the larger capillary was transferred to a large centrifuge and spun at high speed for 15 minutes. The protein precipitated by the acid was thus packed in the bottom of the tube, leaving the supernatant clear. The latter was then drawn off by means of a small suction needle made of the smaller size tubing. The larger tube, containing only the packed protein, was placed in a hot air oven and dried at 110°C. for 15 hours. It was then reweighed, the weight of the dried protein being obtained by difference.

The percentage of protein in the extracellular fluid is compared with the percentage of serum protein from the same animal in table 2.

From the results of the few experiments shown in table 2, it is clearly evident that there is considerable protein contained in this extracellular fluid, and that the percentage of extracellular protein is always less than the percentage of protein contained in the serum of the same animals.

Exactly comparable figures do not exist in the literature, but values for closely related fluids have been obtained. Ayer and Foster (1922) have shown that there is 0.2 per cent to 0.4 per cent protein in spinal fluid; Wunschendorff (1925) estimated the protein in the spinal fluid of man as 0.0187 per cent, and of ascitic fluid from the horse as 1.562 per cent; Churchill, Nakazawa and Drinker (1927) analyzed the protein from frog

lymph and found it to vary from 0.29 per cent to 2.17 per cent, with an average of over 1.0 per cent; Hastings, Salvesen, Sendroy and Van Slyke (1927) showed values of 2.76 per cent for the protein content of human ascitic fluid and 3.00 per cent for edema fluid; Conklin (1930) reported values of 0.66 per cent to 2 per cent for frog lymph; Wells (1932) analyzed the lymph from the lacteals of dogs and reported an average protein content of 2.97 per cent; White, Field and Drinker (1933) obtained values of 0.5 per cent to 1.52 per cent in the lymph from a dog's foot; and Drinker and Field (1933) reported values of 2.87 per cent from lung lymph and 3.79 per cent from the kidney lymph of the dog.

Using the average values for extracellular and serum protein shown in table 2, and Van Slyke's (1926) figure of 12 milliequivalents per litre for

TABLE 2

Protein content of frog extracellular muscle fluid and blood serum

VOLUME OF SAMPLE	WEIGHT OF SAMPLE	WEIGHT OF DRIED PROTEIN	EXTRACELLULAR PROTEIN	SERUM PROTEIN
cu. mm.	*mgm.*	*mgm.*	*per cent*	*per cent*
	1.1	0.019	1.73	
	0.9	0.004	0.44	
1.34	1.4	Lost		
1.30	1.3	Lost		
1.22	1.2	0.009	0.75	
1.26	1.3	0.031	2.38	
1.28	1.3	0.046	3.54	5.05
	1.2	0.006	0.50	3.50
	1.0	0.009	0.90	5.28
	0.9	Lost		4.79
	1.2	0.007	0.58	2.40
	1.4	0.041	2.93	4.35
Average			1.53	4.23

the alkali-binding power of plasma, a chloride ratio was calculated on the assumption of a Donnan equilibrium, according to Van Slyke's (1926) formula,

$$r = \sqrt{\frac{Cl'}{Cl' + P'}}$$

where Cl' = chloride in serum in milliequivalents per litre of water and P' = the alkali-binding power of plasma in milliequivalents per litre. This ratio gave a value of 0.975 for chloride as compared with the figure of 0.993 shown by direct analysis in table 1.

Ratios of chloride in serum to chloride in other body fluids have been obtained, by direct analysis, as follows: for edema fluid, 0.98 (Van Slyke,

1926); for ascitic fluid, 1.007 and for edema fluid, 0.991 (Hastings, Salvesen, Sendroy and Van Slyke, 1927); and for cerebrospinal fluid, 0.82 (Dailey, 1931). It would appear from these ratios that the fluid which was collected in the experiments reported in this paper compares more nearly with edema fluid than any of the other related fluids of the body.

It is hoped that further study of this fluid may be made to characterize it more completely, and that the method of extraction which has been described may be useful in studying fluid samples from other muscles, as well as from tissues other than muscle.

SUMMARY

Clear, straw-colored fluid can be collected from the extracellular spaces of frog muscle in small capillary tubes.

Histological examination shows that the capillary tubes neither tear nor penetrate the muscle fibres, but are confined within the extracellular spaces.

Chloride analysis of this fluid shows that the ratio, serum chloride to extracellular fluid chloride, equals 0.993 ± 0.010.

Erythrocyte counts of the fluid show that it is not collected from broken blood vessels lying between the fibres.

Chloride analyses of the muscles from which the extracellular samples were drawn show that the fluid comprises 19.6 per cent of the weight of the muscle, assuming that all the chloride is extracellular.

Measurements by capillary colorimetry show the pH of the fluid to be the same as that of blood (7.2–7.4).

Gravimetric analysis of the protein of the fluid shows an average of 1.53 per cent as compared with serum protein of 4.23 per cent from the same animals.

On the basis of the protein content, the ratio, serum chloride to extracellular fluid chloride, was calculated on the assumption of a Donnan equilibrium. The value obtained was 0.975 as compared with the experimentally determined value of 0.993.

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THE MECHANISM OF REGULATION OF THE BLOOD SUGAR BY THE LIVER[1]

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That the liver is essential for the maintenance of the level of blood sugar was demonstrated adequately by the early studies on the dehepatized animal. Because of the complexity of the problem, there has been much speculation concerning the mechanism responsible for the normal constancy of the blood sugar level and for the dextrose tolerance curve after the administration of sugar. From the studies of Soskin and his co-workers (1, 2, 3, 4, 5) on the dextrose tolerance curve under various experimental conditions, it would appear that whenever the blood sugar tends to rise above the normal level, the liver responds by diminishing its output of sugar to the blood. The stimulus which elicits this hepatic inhibitory response is the blood sugar itself, and the threshold of stimulation of the hepatic mechanism in a particular animal coincides with the level of blood sugar which that animal habitually maintains. It is suggested that this mechanism is chiefly responsible for the characteristic dextrose tolerance curve when sugar is administered to the normal animal. The influx of exogenous sugar into the blood stream raises the level of the blood sugar above the threshold of stimulation of the homeostatic mechanism. The liver promptly curtails the supply of sugar which it has been pouring into the blood. The exogenous sugar thus temporarily replaces the supply from the liver. Utilization and storage rapidly return the blood sugar toward its normal level, whereupon the liver resumes its secretion of sugar.

This conception was based on indirect evidence. The facilities of the Institute of Experimental Medicine of The Mayo Clinic made it possible to obtain direct proof of the operation of this homeostatic mechanism of the liver.

METHODS. From one to four months prior to our experiments, a two-stage ligation of the posterior vena cava, just below the liver, was performed on the dogs to be used. This rerouting of the blood from the caudal

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end of the body, back to the heart through a newly developed collateral system, enabled us to use the blood flow in the thoracic portion of the inferior vena cava as a measure of the total blood flow through the liver. This method was adopted in preference to measurement of the blood flow through the portal vein and hepatic artery, after preliminary experiments had shown that the latter vessel presented anatomic and physiologic difficulties to our measurement of blood flow. The total blood flowing through the liver was divided into its venous and arterial components, by measuring the rate of inflow through the portal vein and by subtracting this value from the rate of total outflow in the thoracic portion of the posterior vena cava. By this method the blood flow in the hepatic artery

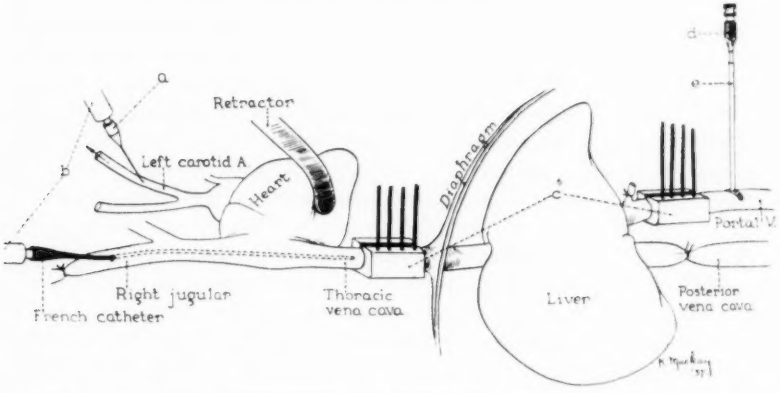

Fig. 1. Diagrammatic representation of procedure. The actual experiments were performed with chest and abdomen closed. The retractor on the heart was used by the artist, but not by the authors. *a*, Hypodermic needle for withdrawing samples of arterial blood; *b*, glass syringes; *c*, thermostromuhr units; *d*, long, flexible needle for use with London cannula, and *e*, modified London cannula.

was obtained. The drainage from the diaphragmatic veins was ignored in these experiments. We considered the possibility that a coincident change in the volume of the liver due to storage of blood might render this estimation of blood flow in the hepatic artery inaccurate. But this was excluded as a significant error by the length of our experiments, and by the magnitude of blood flow through the liver as compared to the volume of blood that the liver is capable of storing.

The procedure employed for this study is illustrated in figure 1. Under sodium amytal anesthesia and with the necessary surgical precautions a thermostromuhr unit (6, 7) was placed on the thoracic portion of the inferior vena cava just above the diaphragm, after which the chest was closed securely so that the animal could breathe naturally. Another unit was

placed on the portal vein as close to the liver as possible. A modified London cannula (8) was sewed to the portal vein just caudad to the thermostromuhr unit. The abdomen was closed, and the right external jugular vein and the left carotid artery were exposed in the neck. It was not necessary to disturb the animal for our procedures during the remainder of the experiment.

The cannula employed differed from the original London cannula in the longer and semicircular plate for attachment to the blood vessel. We found this form more suitable than the original for acute experiments, in which the vein and cannula are not fixed by fibrous tissue. A somewhat similar modification has been described independently by Tsai (9).

At appropriate intervals during the experiments, three samples of blood for estimation of blood sugar (10) were drawn simultaneously by the following methods: Portal blood was obtained by inserting a long, flexible needle into the portal vein through the London cannula mentioned previously. Samples of blood were obtained from the thoracic portion of the inferior vena cava just above the thermostromuhr unit by inserting a French type of urethral catheter into the right external jugular vein and through the innominate vein, superior vena cava and right auricle into the thoracic portion of the inferior vena cava. When the dog is lying on his back with the head extended, the direction of these vessels is such that the catheter usually meets no obstruction, and is not diverted in any other direction. However, care must be taken to prevent air from rushing into the right auricle of the heart. To avoid the formation of intravascular blood clots the catheter was not allowed to remain in place, but was inserted and withdrawn each time a sample of blood was required. Arterial blood was drawn by inserting a fine, hypodermic needle through the wall of the exposed but unobstructed left common carotid artery.

Results. The quantity of sugar entering and that leaving the liver at any given time were calculated in milligrams per minute by correlating the rate of blood flow through the liver with the simultaneously determined values for blood sugar in the inflowing and outflowing blood respectively. The difference between the rates at which sugar was entering and leaving the liver indicated the direction and magnitude of the movement of sugar. These observations were repeated at intervals of fifteen minutes for as long as five hours.

The amount of sugar entering the liver in the portal vein and that entering in the hepatic artery were determined separately from the blood flow and level of blood sugar in the respective vessels. This was important, not only because the level of the blood sugar in the two blood vessels often differed considerably, but especially because we found, in agreement with Schwiegk (11), that the portion of the total blood flow to the liver carried by each vessel varied greatly in different animals, and from time to time in

the same animal. It is, therefore, not permissible to use the rate of blood flow or the level of blood sugar in either vessel as an index of the total inflow of blood or of the entry of blood sugar into the liver.

It is of interest briefly to compare the rates of blood flow through the liver obtained with the thermostromuhr under our experimental conditions, with those previously obtained by use of a mechanical flowmeter. Schmid (12) reported that the total blood flow through the liver of the cat averaged about 54 cc. per 100 grams of liver per minute. For the dog, Burton-Opitz (13) found the average flow through the liver to be about 85 cc. per 100 grams of liver per minute. In the experiments of Macleod and Pearce (14) the total blood flow through the livers of dogs ranged from 64 cc. to 144 cc. per 100 grams of liver per minute. Our results resembled those of Macleod and Pearce. During the control periods of our experiments, before sugar was administered, the average total rate of blood flow through the liver varied in different animals from 40 to 160 cc. per 100 grams of liver per minute. After injection into the femoral vein of 1.75 grams of dextrose per kilogram of body weight in a 30 per cent solution in five minutes, the hepatic blood flow usually increased for a few minutes, but by the time the first complete set of determinations was made, fifteen minutes after the termination of the injection, the blood flow had usually returned to its rate before the injection. In different animals, the portal vein or the hepatic artery sometimes carried as much as 90 per cent or as little as 10 per cent of the total amount of blood entering the liver. Although such large differences in proportionate flow were the exception rather than the rule, smaller reciprocal variations frequently occurred during the course of an experiment, while the total outflow of blood from the liver remained constant. The intravenous administration of the solution of dextrose did not alter these proportions in any consistent manner.

The great mass of data involved precludes the tabular presentation of all our results. Table 1 in which are given the results in one experiment is illustrative of all. But even in this table the data are not complete, since the rate of blood flow is given only when samples of blood were taken, and the duplicate determinations of blood sugar which were made to check our analyses are not included. This is by no means the most technically perfect of our experiments, but is presented because it happens to show a number of incidental phenomena which were observed singly or occasionally in the other experiments. Note the variation between the output and intake of sugar by the liver during the control period, the output being predominant. Note the cessation of the net hepatic output of sugar following dextrose administration, and the sustained intake of sugar lasting one hour. The significance of this rate of sugar intake as regards the disposal of the injected dextrose may be judged from the fact that, even fifteen minutes after the termination of the injection when the values of blood

sugar had already fallen from their initial peaks, the liver was still retaining sugar at a rate of more than 0.5 gram per minute. Note that the resumption of sugar output coincided with the return of the portal and hepatic blood sugar levels to their normal control values. These blood sugar levels then show the characteristic temporary swing below the previous

TABLE 1

*Data on blood flow, blood sugar, and intake and output of sugar by the liver in one animal**

TIME	INTAKE							OUTPUT			NET OUTPUT OF SUGAR	NET INTAKE OF SUGAR
	Portal vein			Hepatic artery				Vena cava				
	Blood sugar	Blood flow	Intake of sugar	Blood sugar	Blood flow	Intake of sugar	Total intake of sugar by liver	Blood sugar	Blood flow	Total output of sugar		
minutes	*mgm. per 100 cc.*	*cc. per minute*	*mgm. per minute*	*mgm. per 100 cc.*	*cc. per minute*	*mgm. per minute*	*mgm. per minute*	*mgm. per 100 cc.*	*cc. per minute*	*mgm. per minute*	*mgm. per minute*	*mgm. per minute*
0	83.3	254	211.6	85.4	174	148.6	360.2	89.2	428	381.8	21.6	
15	83.3	267	222.4	83.3	209	174.1	396.5	80.0	476	380.8		15.7
30	78.2	269	210.4	78.2	196	153.3	363.7	89.0	465	413.9	50.2	
40–45	33 grams of dextrose in 30 per cent solution given intravenously											
60	444.4	307	1364.3	417.0	358	1492.9	2857.2	348.6	665	2318.2		539.0
75	272.0	271	737.1	262.8	203	533.5	1270.6	254.6	474	1206.8		63.8
90	166.6	265	441.5	153.8	133	204.6	646.1	133.3	398	530.5		115.6
105	98.0	262	256.8	97.0	94	91.2	348.0	97.0	356	345.3		2.7
120	67.5	247	166.7	74.6	92	68.6	235.3	83.3	339	282.4	47.1	
135	64.1	234	150.0	68.0	98	66.6	216.6	76.5	332	254.0	37.4	
150	62.5	227	141.9	68.9	120	82.7	224.6	81.3	347	282.1	57.5	
165	64.9	235	152.5	76.9	112	86.1	238.6	93.4	347	324.1	85.5	
180	78.1	241	188.2	81.3	89	72.4	260.6	70.2	330	231.7		28.9
183	Cyanosis noted following difficulty in obtaining the last sample of blood from the vena cava and probable entry of air into right auricle											
195	70.9	317	224.8	114.2	83	94.8	319.6	306.4	400	1225.6	906.0	
210	256.4	243	623.1	266.6	164	437.2	1060.3	310.0	407	1261.7	201.4	
227	151.4	228	345.2	151.4	192	290.7	635.9	224.6	420	943.3	307.4	

* Weight of dog 18.9 kgm. Weight of liver 655 grams.

control values, during the time taken by the liver to adjust its output accurately to the requirements of the organism. As regards the rates of blood flow, note the large variations at different times during the experiment, in the proportions of the total inflowing blood carried by the portal vein and hepatic artery respectively. Finally, it may be seen that the technical difficulty which caused some anoxemia toward the end of the

experiment, was followed immediately by a relatively tremendous output of sugar from the liver and a consequent steep rise in the blood sugar levels. This accidental observation and others like it, indicate the sensitivity of the liver to abnormal conditions, and confirm the satisfactory physiologic status which prevailed during most of our experiments, in which the output of sugar from the liver was of an entirely different order of magnitude.

The result of administration of a single dose of sugar to another dog is illustrated in figure 2. Like the experiment reported in table 1, a reversal of movement of sugar from a greater output to a greater intake occurred

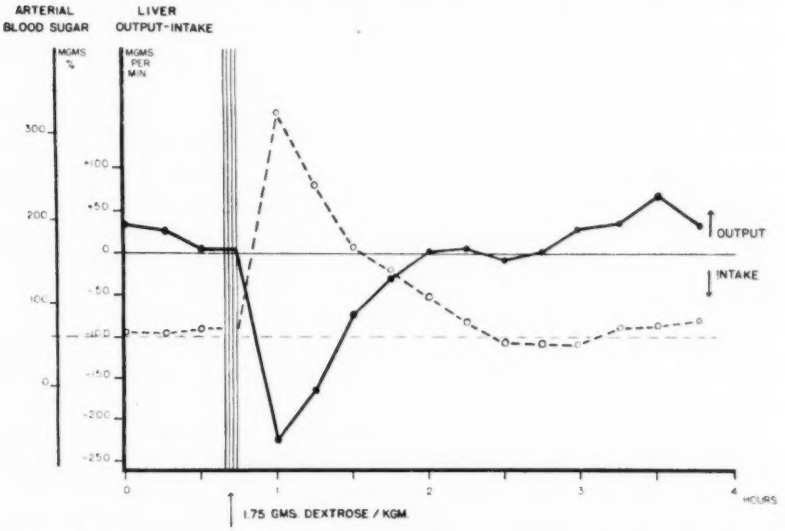

Fig. 2. Effects of the intravenous injection, of five minutes' duration, of 1.75 grams dextrose per kilogram of body weight. Note particularly the period which follows the large retention of sugar by the liver. There is no intake of sugar and the liver does not immediately resume its output. During this period the level of sugar in the arterial blood falls below its original control value, and does not return to normal until after the liver has resumed its output.

following administration of dextrose. But it should be noted that throughout the second hour after administration of sugar the liver was neither retaining nor excreting sugar. It is clear that, following the disposal of most of the sugar administered in this experiment, hepatic storage of sugar ceased while the liver tarried before resuming its supply of sugar to the blood. During this interval the level of sugar in the arterial blood of the animal fell below its original control level and was not restored to normal until after the liver has resumed its output. The inhibition of the hepatic secretion of sugar is, therefore, a real and separate phenomenon from

storage of sugar. This is demonstrated also under different conditions, during the constant injection of smaller amounts of dextrose (fig. 3).

Figures 3 and 4 are representative of a group of experiments in which a comparison of the effect of the administration of different amounts of sugar was simplified by the use of a constant injection instead of a single large dose. The liver responded more or less in proportion to the amount of sugar administered. The constant prolonged injection of 0.5 gram per kilogram of body weight per hour (fig. 3) inhibited the output of sugar, but for some reason, probably related to the physiologic state of the liver as well as to the rate of administration, storage did not occur. Under these circumstances the level of the blood sugar rose somewhat before a new level was established. The prolonged constant injection of 0.75 gram per kilogram of body weight per hour (fig. 4) caused storage as well as inhibition of output. The animal was able, therefore, to establish a balance at a lower level for blood sugar in spite of the greater amount of sugar it had to deal with. In another experiment, the record of which has been omitted to conserve space, the prolonged constant injection of 1.5 grams of dextrose per kilogram of body weight per hour could not be compensated for in spite of both an inhibition of output and a large storage of sugar. In this experiment, although the output promptly ceased and the intake varied from about 100 to 200 mgm. per minute throughout the period of injection, the arterial blood sugar gradually rose from its initial level of 70 mgm. per cent to a final value of 262 mgm. per cent at the end of the injection period.

Comment. The secretion of sugar into the blood by the liver of the fasting animal was demonstrated by the brilliant pioneer work of Claude Bernard (15). He also found that glycogen accumulated in the liver after feeding of carbohydrate, although he was inclined to believe that this was not a result of the direct conversion of the ingested sugar into glycogen. Bernard and his contemporaries made extensive use of the method of comparing the sugar contents of the inflowing and outflowing blood of the liver. The inadequate state of knowledge concerning methods of estimating the level of sugar, the phenomenon of glycolysis, and so forth, however, resulted in controversial conceptions of liver function and eventually obscured the significance of Bernard's dictum, that "The normal blood sugar level is the result of a precise equilibrium between the processes of anabolism (sugar formation in the liver) and catabolism (sugar utilization in the tissues)."

More recent comparisons of the sugar contents of the inflowing and outflowing blood of the liver during the absorption of sugar from the gastrointestinal tract (8, 16–20) or during the intravenous administration of sugar (21), have largely confirmed Bernard's original conception. But we cannot accept the calculations by which some of the later workers have

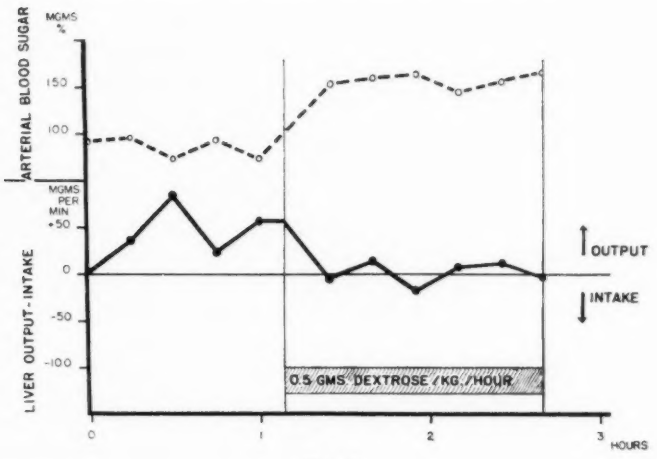

Fig. 3

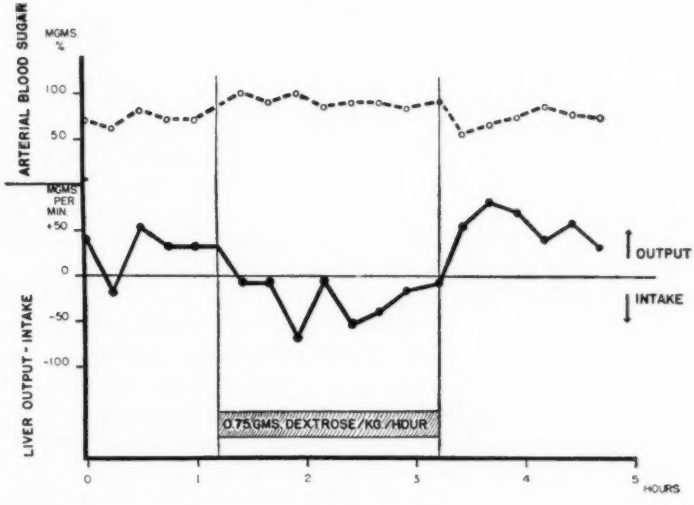

Fig. 4

Figs. 3 and 4 (to be grouped together). Comparison of the effects of different amounts of sugar administered by prolonged constant intravenous injection. In these, and in other experiments not shown, the liver intake of sugar is more or less proportionate to the rate of sugar administration. The lowest rate of injection (fig. 3), however, results in a suppression of the output of sugar from the liver, without any storage of sugar. In this case the constant injection of 0.5 gram of dextrose per kilogram of body weight per hour was just sufficient to replace the previous endogenous supply of blood sugar from the liver, and there is little change in the arterial blood sugar level.

attempted to give their results quantitative significance. In view of our present work it is evident that no constant or average hepatic blood flow can be assumed for purposes of calculation. It is also clear that, in the absence of measurements of blood flow, differences in the blood sugar between the inflowing and outflowing blood can indicate little more than the direction of movement of sugar into or out of the liver.

The observations which Soskin and his co-workers made on the inflowing and outflowing blood of the liver, in normal and depancreatized dogs (1) were subject to the same limitations as those of other investigators. Their coincidental studies of the dextrose tolerance curve, however, led them to differentiate between the storage of incoming carbohydrate and the suppression of output of sugar. This, in turn, gave rise to their interpretation of the observed hepatic activity as a homeostatic mechanism for the maintenance of a constant level of blood sugar, as described in our introductory remarks. The quantitative results, obtained in our study by correlating the blood sugar difference with the blood flow, yield direct proof for the interpretations and conclusions of Soskin and his co-workers. The reader is referred to the papers by these investigators for a consideration of the significance and implications of this hepatic mechanism.

SUMMARY AND CONCLUSIONS

The rate of blood flow through the liver and the arterial and venous components of the total hepatic blood flow were observed by means of the thermostromuhr in specially prepared dogs. The output or intake of sugar by the intact liver in situ was calculated in milligrams per minute by correlating the rates of blood flow with the simultaneously determined content of blood sugar of the inflowing and outflowing blood.

The movement of sugar out of, or into, the liver was observed during control periods and after the intravenous administration of sugar. Prolonged constant injections of dextrose as well as single large doses (dextrose tolerance tests) were used. During the control periods the liver was observed to secrete sugar into the blood. The administration of dextrose was invariably followed by cessation of excretion of sugar by the liver and by retention of a portion of the incoming sugar. Inhibition of the output of sugar was observed in the absence of storage of sugar, following the administration of certain smaller doses of sugar and at certain intervals after the administration of large doses. At these times, the level of the animal's arterial blood sugar temporarily fell below the original control values and remained low until resumption of secretion of sugar by the liver restored it to its previous levels.

Our results yielded direct and quantitative evidence of the homeostatic regulation of the level of blood sugar by the liver, a mechanism for which only indirect evidence was previously available.

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